

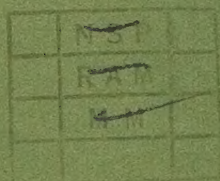
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## Sugar Absorption, Callose Formation and the Growth Rate of Pollen Tubes

JAROSLAV TUPÝ

Department of Plant Physiology, Institute of Biology, Czechoslovak Academy of Sciences, Praha

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### Souhrn

Závislost rychlosti růstu pylových láček jabloně na čase je při 25° C v 10% roztoku sacharosy bez boru či s borem (0,001%  $H_3BO_3$ ) prakticky lineární po dobu 6 hod., u pomaleji rostoucího pylu *Nicotiana alata* dochází k poklesu růstové rychlosti teprve po 10 až 15 hod.

Většina sacharosy v kultivačním roztoku je pylovými láčkami invertována. Rychlost této reakce se s časem až do vyčerpání substrátu zvyšuje. S poklesem koncentrace sacharosy na 5 %, ke které dochází především v důsledku její inverze, klesá rychlost růstu pylových láček. V roztoku samotného invertu je klíčení pylu nepatrné. To znamená, že inverzí je sacharosa vzhledem k možnosti jejího využití pylovými láčkami znehodnocována.

Rostoucí pylové láčky přijímají z kultivačního roztoku sacharosu. Množství přijímané sacharosy klesá přibližně stejně jako rychlost jejich růstu. Z toho lze usoudit, že hromadění kallosy v pylových láčkách během zpomalování jejich růstu není způsobeno zvýšením podílu glukosy nevyužité ke stavbě jejich blan. Jestliže dále nebyla při omezení růstu láček zjištěna v kultivačním roztoku změna v poměru glukosy a fruktosy, vyplývá z toho, že nejspíše dochází během snižování růstové rychlosti a zvýšeného hromadění kallosy k poklesu prodýchávaného podílu glukopyranosové složky přijaté sacharosy.

Proporcionálně se stimulací růstu láček podporuje u nich bor příjem sacharosy. Je tedy možné, že s metabolismem glycidů souvisí princip stimulačního působení boru na růst. Vzhledem k tomu, že růst láček je borem urychlován ještě v době, kdy rostou převážně nebo zcela na účet rezervních látek v pylovém zrnu a ke známému stimulačnímu působení boru v destilované vodě, nemusí zde jít primárně o translokaci glycidů do láček, nýbrž spíše o rychlost jejich metabolismu.

## Summary

1. The growth rate of apple pollen tubes at a temperature of 25° C in 10% sucrose solution, with or without boron (0.001%  $\text{H}_3\text{BO}_3$ ), shows a practically linear relation to time for 6 hours; *Nicotiana alata* pollen tubes, which are slower in growth, show a decrease in growth rate after 10 to 15 hours.

2. The greater part of the sucrose in the culture solution is inverted by the pollen tubes. The rate of this reaction increases with time until the substrate is exhausted. When the sucrose concentration drops to 5%, chiefly as a result of the inversion process, the growth rate of the pollen tubes decreases.

3. The growing pollen tubes absorb sucrose from the culture solution. Exogenous utilization of sucrose was evident in the case of apple pollen after 3 hours, in *Nicotiana* pollen after 6 to 8 hours of incubation.

4. In view of the close correspondence between the decrease in the intensity of sucrose absorption by the pollen tubes and the decrease in their growth rate, it may be concluded that the accumulation of callose in pollen tubes while their growth rate is decreasing is not due to any rise in the proportion of glucose not utilized for cell-wall formation. Further, if no change in the glucose: fructose ratio is observed in the culture solution at the time when pollen tube growth is slowed down, it follows that reduction in growth rate and increase in callose accumulation is most probably accompanied by a drop in respiration of glucopyranose component of the absorbed sucrose.

5. Boron encourages sucrose absorption proportionally to the stimulation of pollen tube growth. It is, therefore, possible that the stimulatory effect of boron on growth is connected with carbohydrate metabolism. In view of the fact that pollen tube growth is accelerated by boron at the time when the tubes are still drawing to a great extent or exclusively on the reserve substances in the pollen grain—and in view of the well-known stimulatory effect of boron in distilled water—it follows that the main factor is not necessarily the translocation of carbohydrates into the pollen tubes, but that it is rather a question of the rate of their metabolism.

## Introduction

In studying the interaction of germinating pollen, or growing pollen tubes, and the pistil tissues, consideration is often given to their interaction in the sphere of carbohydrate metabolism. The physiological basis of incompatibility, for example, is sometimes linked with factors of pollen tube nutrition by the style tissues (POLYAKOV 1949, ESSER 1953, EUE 1953, LINSKENS 1955, BREWBAKER 1957). Elucidation of the main questions regarding pollen tube nutrition is one of the prerequisites for the successful explanation of such relationships.

In spite of the fact that a number of data have already been assembled providing evidence that the nutrition of pollen tubes is dependent on the environment (GREEN 1894, BRINK 1924, SCHOCH-BODMER and HUBER 1947,

STRAUB 1947, LINSKENS 1955, O'KELLEY 1955, IWANAMI 1956a, 1956b, HELLMERS and MACHLIS 1956), sugar absorption by pollen tubes is sometimes denied (EHLERS 1951, VISSER 1955). The function of carbohydrates in a solution in which pollen is germinating is then regarded as being only the regulation of water absorption (VISSER 1955). Particularly in view of the last-mentioned work, and in view of the fact that utilization of sugar from the surroundings had only been demonstrated with certainty in the case of *Tecoma radicans* pollen (O'KELLEY 1955), it was necessary to investigate this matter experimentally. \*)

Glucose is laid down in pollen tubes in the form of callose. The physiological basis of the formation of this polyglucoside is regarded as being the limited capacity of pollen tubes to utilize the glucopyranose component of metabolised sucrose during respiration. This serves to explain the increased accumulation of this polysaccharide in slow-growing incompatible pollen tubes (TUPÝ 1959). This can be due to the decrease in absorption of sugars from the surrounding medium being less than the decrease in the growth rate of the pollen tubes, i.e. the proportion of glucopyranose not utilized for cell-wall formation is increased and is then laid down to an increasing extent in the form of its polymer callose. In testing the correctness of the above assumption it was necessary to examine not only this relationship, but also the question of whether the proportions of the components of metabolized sucrose which are absorbed are not changed during retarded growth.

In order to enable the relation between the rate of absorption of sugars and growth rate to be determined in this connection, attempts were made to alter this factor in a suitable manner during the experiments. Boron was used for this purpose, because of its well-known stimulatory effects on pollen tube growth. This means that the physiological basis of this influence was also followed, this being still an unsolved question. In addition to the possible relationship between the stimulatory effect of boron and the increased absorption of sugars (O'KELEY 1957), it is also assumed that its action is linked with the formation of a complex of boric acid with an unknown substance in the pollen, without any absorption of carbohydrates from the surrounding medium (VISSER 1955).

### Materials and Methods

The most recent work on the nutrition of pollen tubes and the stimulatory effect of boron was carried out with apple pollen (VISSER 1955). Since the formation of callose in pollen tubes in connection with their incompatibility inhibition was also followed in apple pollen (TUPÝ 1959), this material was used for subsequent experiments. Pollen of the variety Golden Pearmain was chosen for its very good germination qualities. It was kept for 9 months before use in small, closed, glass vessels at a temperature of about  $-15^{\circ}\text{C}$ . In the course of a year at this temperature no reduction in its approximately 95% germination percentage was observed. The absorption of sugars from the surrounding medium in relation to the growth rate of the pollen tubes was

\*) Further evidence on the exogenous substrate utilisation by growing pollen tubes was made using *Lilium* and *Petunia* plants. It was shown that during their growth the pollen tubes take up material from the style (LINSKENS and ESSER 1959). We have been acquainted with this work after the manuscript was received by the editors.

also followed for pollen of *Nicotiana alata* LINK et OTTO; this pollen had a germination percentage of 60% and was kept as described above for 5 months.

Pollen to the amount of 5 mg. was cultivated in small porcelain dishes (35 × 24 mm.) placed in a moist chamber at 25° C. The volume of culture solution—10% (v/w) sucrose solution without boron and with boron (0.001% (v/w)  $H_3BO_3$ )—was 0.5 ml. The periods of cultivation were 3, 6, 10, 15 and 20 hours. The solutions, dishes and moist chambers were sterilized before use. No method for the removal of microorganic contamination of pollen is known as yet (HELLMERS and MACHLIS 1956). Therefore, 20 mg. of pollen was suspended in 2 ml. of 10% sucrose solution. Following separation of the pollen by filtering, the filtrate was used as a control solution.

When the incubation period had ended, the culture solution was poured off, it was cleared of the few remaining pollen grains or pollen tubes by centrifuging, mixed with an equal volume of 96% ethyl alcohol and placed in a refrigerator until used. A small quantity of germinated pollen was transferred from the dish to a slide. Part of it was stained with resorcin blue, prepared by ESCHRICH's method (1953), and used for determining the size of the callose plugs; the other part was stained and kept in a 1% solution of aniline blue in lactophenol so that it should be available in case of need for further measurement of the lengths of pollen tubes. In each sample 50 pollen tubes were measured and the lengths of callose plugs were recorded in 20. The results of these measurements were dealt with by the usual statistical methods.

Reducing sugars were quantitatively determined following chromatographic separation on Whatman No. 1 paper in the system n-butanol-acetic acid-water (4 : 1 : 5). A 2% alkaline methanolic solution of 2-3-5-triphenyltetrazoliumchloride was used for detection; the formazan produced over a period of 20 min. in an atmosphere saturated with water vapour at a temperature of 75° C was then, following drying of the chromatogram for 30 min. at 70° C, eluted with methanol and subjected to colorimetric analysis (WALLENFELS, BERNT, LIMBERG 1953, FISCHER and DÖRFEL 1954). Total sugars in the culture solution were determined following hydrolysis with oxalic acid using Somogyi-Nelson's method (NELSON 1944). An error of  $\pm 5\%$  must be reckoned with in the chromatographic determination of sugars; the error with Somogyi-Nelson's method is  $\pm 2\%$ . Glucose and fructose were determined twice in all cases; alternation in the total amount of sucrose was calculated from three (for apple pollen) or two (for *Nicotiana*) determinations.

## Results

### 1. Growth of pollen tubes

The results of measurements of the lengths of pollen tubes in the different time variants of the experiments are set out in Table 1. When they are plotted

Table 1. Length of Apple and *Nicotiana* Pollen Tubes in Artificial Cultures

| Time of cultivation | Average length of pollen tubes in $\mu$ |                                         |                             |                      |                                         |                             |
|---------------------|-----------------------------------------|-----------------------------------------|-----------------------------|----------------------|-----------------------------------------|-----------------------------|
|                     | <i>Malus</i>                            |                                         |                             | <i>Nicotiana</i>     |                                         |                             |
| hours               | 10% sucrose solution                    | 10% sucrose solution + 0.001% $H_3BO_3$ | Significance of differences | 10% sucrose solution | 10% sucrose solution + 0.001% $H_3BO_3$ | Significance of differences |
| 3                   | 378 $\pm$ 3.19.57                       | 472 $\pm$ 3.22.34                       | P < 0.01                    | 113 $\pm$ 3.12.58    | 128 $\pm$ 3.15.34                       | P > 0.05                    |
| 6                   | 729 $\pm$ 3.55.96                       | 985 $\pm$ 3.81.00                       | P < 0.01                    | 177 $\pm$ 3.14.81    | 231 $\pm$ 3.18.60                       | P < 0.05                    |
| 10                  | 871 $\pm$ 3.48.46                       | 1289 $\pm$ 3.56.90                      | P < 0.01                    | 298 $\pm$ 3.17.97    | 418 $\pm$ 3.22.34                       | P < 0.01                    |
| 15                  | 1012 $\pm$ 3.46.64                      | 1539 $\pm$ 3.90.18                      | P < 0.01                    | 405 $\pm$ 3.24.35    | 567 $\pm$ 3.41.21                       | P < 0.01                    |
| 20                  | —                                       | —                                       | —                           | 546 $\pm$ 3.37.46    | 668 $\pm$ 3.43.40                       | P < 0.05                    |

Fig. 1. Growth of *Malus* and *Nicotiana* pollen tubes during their artificial cultivation.

ordinate: length of pollen tubes in  $\mu$   
abscissa: time of cultivation in hours

*Malus* 10% sucrose solution ●——●  
10% sucrose solution + 0.001%  $H_3BO_3$  ○——○  
*Nicotiana* 10% sucrose solution ▲——▲  
10% sucrose solution + 0.001%  $H_3BO_3$  △——△

as a graph (Fig. 1) it can be seen that, in the case of apple pollen, the relation of the growth rate of the pollen tubes to time is practically linear during the first six hours in cultures with and without boron. Later, the rate decreases with increasing rapidity. In the case of the more slow-growing *Nicotiana* pollen tubes reduction in growth rate becomes evident after 10 to 15 hours. Following 20 hours of cultivation, the majority of the pollen tubes of both species exhibited a club-shaped swelling of the tips similar to that known in their incompatibility inhibition.

## 2. Inversion of sucrose by pollen tubes

When analysing the culture solution during the growth of pollen tubes the first fact to be determined was the inversion of sucrose. Apple pollen tubes hydrolyse sucrose with greater intensity than the more slow-growing *Nicotiana* pollen tubes (Fig. 2). In both cases a lesser degree of inversion was always obtained in cultures containing boron. The rate of inversion increases with time; it only decreases when the substrate has been considerably exhausted, as in the case of apple pollen. At the time of this drop, i.e. following

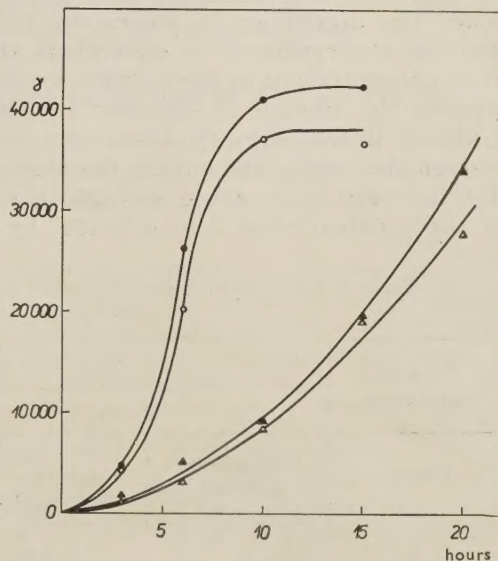
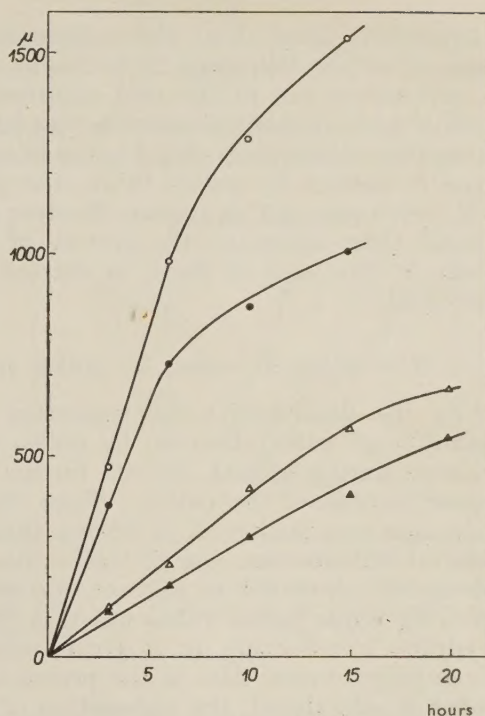


Fig. 2. Inversion of sucrose with *Malus* and *Nicotiana* pollen tubes.

ordinate: amount of inverted sucrose in  $\mu g$ .  
abscissa: time of pollen cultivation in hours.  
Further explanation see Fig. 1.

10 hours of incubation, the culture solution contained only about 0.4% of sucrose, while following 20 hours of cultivation of *Nicotiana* pollen, when a continuous rise in the rate of inversion is evident, its concentration was still 2%. Inversion of sucrose was halted by the removal of pollen tubes from the culture (following 4 hours of cultivation). In view of the rapid hydrolysis of sucrose by pollen tubes, the germination of pollen in 10% (v/w) and 5% (v/w) solution of glucose, fructose and their mixture (1 : 1) was followed. In all these solutions the growth of pollen tubes was considerably slower than in the case of those in sucrose solution, or practically nil (fructose solution).

### 3. Absorption of sugars by pollen tubes

By the quantitative determination of all sugars and calculation of the quantity of water absorbed by pollen tubes on the basis of their increase in volume during growth, it was further possible to estimate the decrease in sugar content of the culture. Since the sucrose concentration of the control solutions was unaltered, it follows that the decrease in sugars in the experimental cultures was due to their utilization by the pollen tubes. The values obtained, converted to sucrose, are set out in Table 2. The more rapidly-growing apple pollen tubes utilize a considerably greater quantity of carbohydrates exogenously in a given period than the more slow-growing *Nicotiana* pollen tubes. Also in the presence of  $H_3BO_3$ , when the growth of pollen tubes is stimulated, the exhaustion of sugars from the cultures is higher in the case of both types of experimental material than from solutions of sucrose alone. The absorption of sugars by both types is considerably more rapid than the absorption of an equivalent amount of water corresponding to the given concentration of the culture solution at the start (Fig. 3). The relation between the amount of absorbed sucrose and the length of the pollen tubes is almost linear (Fig. 4). The curve drawn by extrapolation does not pass through the origin, but crosses the abscissa at the point for pollen tube length of 410 to 440  $\mu$  for apple and 260  $\mu$  for *Nicotiana*. This length is attained by apple pollen after 2 to 4 hours, by *Nicotiana* pollen after 6 to 8 hours.

Table 2. Amounts of Sugars Absorbed by Pollen Tubes in Artificial Cultures, Converted to Sucrose

| Time of cultivation | Amounts of absorbed sucrose in mg. |                                         |                      |                                         |
|---------------------|------------------------------------|-----------------------------------------|----------------------|-----------------------------------------|
|                     | <i>Malus</i>                       |                                         | <i>Nicotiana</i>     |                                         |
| hours               | 10% sucrose solution               | 10% sucrose solution + 0.001% $H_3BO_3$ | 10% sucrose solution | 10% sucrose solution + 0.001% $H_3BO_3$ |
| 3                   | 0                                  | 0.33                                    | 0                    | 0                                       |
| 6                   | 4.46                               | 3.87                                    | 0                    | 0                                       |
| 10                  | 6.48                               | 11.64                                   | 1.97                 | 5.76                                    |
| 15                  | 8.20                               | 11.69                                   | 2.83                 | 7.02                                    |
| 20                  | 11.00                              | 13.89                                   | 7.11                 | 10.95                                   |

This is in agreement with the finding that no or only a little drop in content of sugars in the culture solution was obtained with apple pollen after 3 hours and with *Nicotiana* pollen after 3 and 6 hours.

In the majority of cases less fructose than glucose was found in the culture solutions. During the cultivation of pollen, however, no regular change in

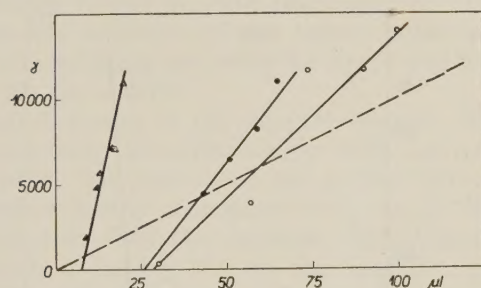


Fig. 3. Relation of the water absorption (abscissa, in  $\mu\text{l.}$ ) to the sucrose utilisation (ordinate, in  $\mu\text{g.}$ ) by *Malus* and *Nicotiana* pollen tubes. Broken line expresses this relation in the case of proportional consumption of sucrose and water. Further explanation see Fig. 1.

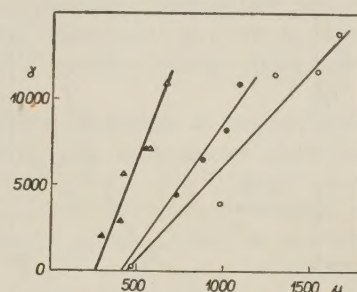


Fig. 4. The relation of the length of pollen tubes (abscissa, in  $\mu.$ ) to the amount of absorbed sucrose (ordinate, in  $\mu\text{g.}$ ). Explanation see Fig. 1.

their relation was obtained. The differences in the decreases of glucose and fructose expressed as percentages of the total decrease of sugars was not significant when calculated as an average for all time-variants.

#### 4. Formation of callose in pollen tubes in relation to their growth rate

The amount of callose was evaluated as a sum of the lengths of all callose plugs in the pollen tube. Since these formations do not start to be laid down until the third to sixth hour of pollen cultivation, it was impossible to make

Table 3. Accumulation of Callose in Apple Pollen Tubes in Artificial Culture

| Period of cultivation | Average length of callose plugs in $\mu$ |                   | Average amount of callose in 1 pollen tube as total length of callose plugs in $\mu$ |                   | Q    |      |
|-----------------------|------------------------------------------|-------------------|--------------------------------------------------------------------------------------|-------------------|------|------|
|                       | 1                                        | 2                 | 1                                                                                    | 2                 | 1    | 2    |
| 6                     | 14.2 $\pm$ 3.1.15                        | 15.5 $\pm$ 3.0.60 | 13.5 $\pm$ 3.1.48                                                                    | 16.9 $\pm$ 3.2.09 | 54.0 | 58.3 |
| 10                    | 21.6 $\pm$ 3.2.23                        | 18.9 $\pm$ 3.1.96 | 26.0 $\pm$ 3.3.51                                                                    | 29.7 $\pm$ 3.2.29 | 33.5 | 43.4 |
| 15                    | 22.9 $\pm$ 3.1.89                        | 27.0 $\pm$ 3.2.09 | 47.2 $\pm$ 3.5.13                                                                    | 79.6 $\pm$ 3.8.91 | 21.4 | 19.3 |

1 : culture = 10% sucrose solution

2 : culture = 10% sucrose solution + 0.001%  $\text{H}_3\text{BO}_3$

$$Q = \frac{\text{average length of pollen tubes in } \mu}{\text{total length of callose plugs in 1 pollen tube in } \mu}$$

a quantitative recording of callose after 3 hours of incubation. In rapidly growing pollen tubes stimulated by boron less callose is laid down in relation to their length than in those grown in solutions of sucrose alone (Table 3).

While the growth rate of the pollen tubes is decreasing the quotient of their length to the amount of callose (given as the length of callose plugs)

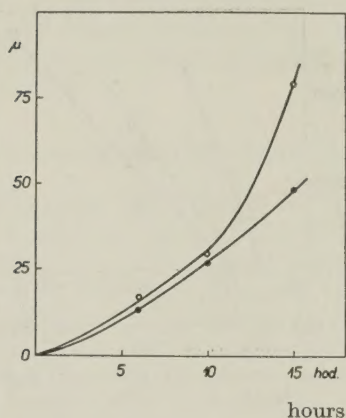


Fig. 5. Relation of the amount of callose in pollen tubes expressed in the length of total callose plugs in pollen tube (ordinate, in  $\mu$ ) to the time of their cultivation (abscissa, in hours). Explanation see Fig. 1.

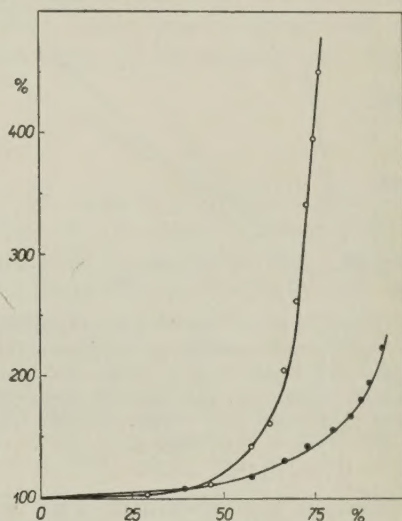


Fig. 6. Relation of the rate of callose accumulation in pollen tubes expressed in % of the increase in callose during 1 hour (ordinate, 100% = one hour increase in callose during first 6 hours of pollen cultivation) to the rate of their growth expressed in % of inhibition (abscissa). Explanation see Fig. 1.

goes down rapidly. In a solution containing  $H_3BO_3$  the rate of accumulation of callose in the pollen tubes rises more rapidly than in cultures lacking boron. After 15 hours of cultivation the relative amount of callose is about the same in both cases. The accumulation of callose is accelerated not only in relation to the length of the pollen tubes, but also absolutely in relation to the time factor (Graph 5). The rate at which it is laid down, expressed as the hourly increase in length of all callose plugs in the pollen tube, is an exponential function of slowing down in growth of the pollen tubes expressed in percentages of inhibition (Graph 6).

### Discussion

The observed inversion of sucrose by pollen tubes merely confirms the results of several authors (LINSKENS 1955, IWANAMI 1956a, 1956b, SCHADE 1957) using different material. However, this reaction had not hitherto been

followed quantitatively during the growth of pollen tubes. The fact that inversion of sucrose is halted when the pollen tubes are removed from the culture indicates that invertase is not released by them into the medium. The splitting of sucrose takes place either within the pollen tubes or is most probably bound to their surfaces, as in the case of yeasts (according to GOTTSCHALK 1958) and the somatic cells of higher plants (HASSID 1958). This is evidently linked up with the increased rate of this reaction during the period when the surfaces of the pollen tubes are being enlarged by growth, and with its differing intensity for apple and tobacco pollen tubes whose respective growth rates differ.

The decrease in the sucrose content of the culture solution is mainly due to inversion. Simultaneously with the decrease in the amount of sucrose, however, the growth of the pollen tubes is slowed down. Since apple and *Nicotiana* pollen germinate very badly in solutions of inversion products, it is probable that the decrease in the growth rate of pollen tubes in sucrose solution is mainly due to the inversion process. With both types of pollen, growth is slowed down when the sucrose content of the medium drops to 5%. This is in agreement with the observation that germination of apple and pear pollen is lower in a 5% solution than at the optimal 10% concentration (VISSER 1955). From this aspect, however, the biological significance of so high an invertase activity in pollen tubes is not clear. If under natural conditions in the style the growth rate of pollen tubes does not decrease and, what is more, if pollen tubes from repeated pollination grow normally (STRAUB 1947), it seems probable that the activity of pollen invertase, or perhaps the nature of its action (hydrolysis, synthesis), must be controlled in the style tissue. This also follows from the fact that while the pollen tubes grow through the style there is no rise in the level of monosaccharides there, on the contrary there is a drop (LINSKENS 1955).

The results obtained from the observation of sugar absorption by pollen tubes provide a further direct proof, alongside O'KELLEY'S (1955, 1957) work using *Tecoma radicans* pollen, of the dependence of their carbohydrate nutrition on the medium. Thus, sugars have, besides their probable function of regulating water absorption by pollen tubes (VISSER, 1955), a further significance as a nutrient.

It follows from the relation between the length of the pollen tubes and the amount of absorbed sucrose that the slowing down of both processes during the later phase of growth is practically equal. The limited growth of the pollen tubes is, however, rather a linear function of the amount of absorbed sucrose than the reverse. The absorption of this sugar is then limited by a marked drop in its level, mainly as a result of the hydrolytic decomposition brought about by pollen invertase. The increase in the formation of callose in inhibited pollen tubes, based on the assumed principle of the limited capacity of pollen tubes to use the glucose component of metabolised sucrose for respiration, has been explained by the increase in the proportion of unused glucose, which would have been used up for the formation of pollen tubes during rapid growth (TUPÝ 1959). If, however, the amount of absorbed sucrose falls to a degree equivalent with the reduction of growth rate, it follows that the increased accumulation of callose in the pollen tubes whose growth is limited

cannot be explained in this manner. And further, if during limited growth of the pollen tubes no change in the ratio of glucose to fructose was found in the culture solution, it follows that most probably a reduction takes place in the share of the respired glucopyranose component of the absorbed sucrose during the period of reduction in growth rate and increased accumulation of callose. This would mean that the capacity of pollen tubes to use these forms of sugars for respiration is not constant under the given conditions, but is reduced at the time of limited growth.

In view of the fact that the growth of pollen tubes is primarily dependent on the sucrose content of the culture solution, and not on that of the products of inversion, it must be assumed that they also absorb sugars mainly in the form of saccharose. In accordance with O'KELLEY's (1957) findings, sucrose absorption is accelerated by boron. In the course of pollen cultivation this stimulation is approximately proportionate to the stimulation of the growth rate of the pollen tubes. Thus, it is not excluded that the principle of the stimulatory effect of boron on growth is connected with carbohydrate metabolism. Translocation of carbohydrates into the pollen tubes is not necessarily of primary importance here, it may be rather a question of the rate of their metabolism in view of the fact that pollen tube growth is encouraged by boron at the period when absorption of sugars has not been observed, and in view of the known stimulatory effect of boron in distilled water. Some more recent experiments also show that boron is closely linked in plants with the activity of meristem cells and that the increased translocation of sugars in the presence of boron is rather a consequence of increased cell activity than of the formation of sugar-borate complexes (SKOK 1956, 1957). This is also in agreement with the supposition that boron encourages the movement and utilization of reserve substances in connection with the stimulation of the activity of adenosin-triphosphate (DANIEL and VÁROCZY 1957).

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Address: Ing. Jaroslav Tupý, Biologický ústav ČSAV, Praha 6 · Dejvice, Na cvičišti 2

## Абсорбция сахара, образование каллозы и скорость роста пыльцевых трубок

Ярослав Тупы

Отделение физиологии растений, Биологический институт Чехословацкой академии наук, Прага

### Резюме

Зависимость скорости роста пыльцевых трубок от времени в 10%-ном растворе сахарозы без бора или же с бором (0,01%  $H_3BO_3$ ) при температуре 25°C является в течение первых 6 часов почти линейной. У более медленно прорастающей пыльцы *Nicotiana alata* падение скорости роста имеет место лишь через 10—15 часов.

Большая часть сахарозы из питательной среды инвертируется пыльцевыми трубками. Скорость этой реакции возрастает пропорционально времени вплоть до полного израсходования субстрата. С понижением концентрации сахарозы до 5%, что происходит прежде всего в результате инверсии, скорость роста пыльцевых трубок падает. В растворе инвертированного сахара прорастание пыльцы происходит лишь в незначительной мере. Это значит, что инверсией возможна возможность использования сахарозы пыльцевыми трубками, повидимому, снижается.

Из питательного раствора растущие пыльцевые трубки принимают сахарозу.

На основании почти полного схождения кривых, обозначающих падение интенсивности потребления пыльцевыми трубками сахарозы и падения скорости их роста можно предположить, что накопление каллозы в пыльцевых трубках по ходу замедления их роста не вызвано тем, что часть глюкозы не используется для построения клеточной оболочки. Кроме того, если при ограниченном росте пыльцевых трубок в питательном растворе не было установлено изменение отношения глюкозы и фруктозы, то из этого вытекает, что при снижении скорости роста и накоплении каллозы имеет место, скорее всего, сокращение количества глюкopiранозы, расходуемой при дыхании.

Пропорционально стимуляции роста пыльцевых трубок бор содействует также повышению количества потребляемой сахарозы. Поэтому не исключено, что стимуляция роста в присутствии бора в принципе связана с действием последнего на обмен углеводов. Так как рост пыльцевых трубок стимулируется бором еще в то время, когда они большей частью или полностью развиваются за счет запасных веществ, содержащихся в пыльцевом зерне, и также учитывая известный факт стимуляционного действия бора в дистиллированной воде, можно предположить, что в данном случае стимуляция первично не связана с перемещением углеводов в пыльцевые трубки, а скорее со скоростью их метаболизма.

## The Effect of Two Mucoproteins on Infection by Tobacco Mosaic Virus

ZDENKO POLÁK

Department of Plant Pathology, Institute of Biology, Czechoslovak Academy of Sciences

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### Souhrn

Studovali jsme inhibiční účinek mucínu izolovaného ze submaxilárních hovězích žláz a mukoproteidu z lidské moče na infekci virem tabákové mosaiky (VTM). Použili jsme metody kvantitativního vyšetření relativní virové koncentrace na listech *Nicotiana glutinosa* L. Oba preparáty byly vyšetřeny jednak ve směsích s infekční šťávou z rostlin *N. tabacum* L. Samsun, ochuravělých žlutým kmenem VTM, jednak s roztokem purifikátu zeleného isolátu VTM.

Inhibiční účinek mucínu na infekci purifikátem VTM dosahuje ve směsi obou komponentů v poměru 1 : 1 76,8 %. Je však natolik tlumen přítomností surové šťávy z tabáku, že ve směsi mucínu s infekční šťávou v poměru 1 : 1 inhibice klesá na 20—35 %. Dále byl srovnáván inhibiční účinek mucínu s účinkem čerstvých slin. Z těchto pokusů vyplývá, že sliny obsahují kromě mucínu ještě jinou inhibiční složku, která na rozdíl od mucínu nemá okamžitou inhibiční účinnost.

Močový mukoproteid je inhibítorem efektivnějším; jeho účinnost je také okamžitá. Ve směsi s infekční šťávou v poměru 1 : 1 je jeho inhibiční účinek 78,7 %. Infekce purifikátem VTM je inhibována téměř stoprocentně. Z těchto pokusů, právě tak jako z výsledků pokusů s mucínem je patrný rozdíl v účinku inhibitorů na infekční šťávu a roztok purifikátu VTM. Studovali jsme proto kombinační schopnost mukoproteidu izolovaného z moče s purifikátem VTM a surovou šťávou ze zdravého tabáku a zjistili jsme, že výsledná infektivita směsi těchto látek závisí na pořadí, v jakém jsou do směsi přidávány. Z našich pokusů vyplývá, že se mukoproteid kombinuje jak s VTM, tak s nedefinovanou látkou obsaženou v surové šťávě z tabáku.

### Summary

The inhibitory effect of mucin on infection with purified TMV in a mixture of the two components in the proportion of 1 : 1 attains 76.8%. Mucin solution tested in the same proportion with infective juice reduces the infectivity of the mixture by only 20—35%. In human saliva compared with mucin as

regards their inhibitory activity there is evidently another inhibitor at work, which does not exert an immediate inhibitory influence as in the case of mucin.

Urinary mucoprotein mixed with infective juice in the proportion of 1 : 1 reduces infectivity by 78.7%. Infection with purified TMV is inhibited by it by almost 100%. All the results mentioned were statistically significant.

The present experiments have further followed the mutual relation between purified TMV, fresh juice from healthy tobacco and mucoprotein. The results show that the infectivity of the mixture of these substances depends on the order in which they are added. It follows from the experiments that mucoprotein also combines with an undefined substance in fresh tobacco juice.

### Introduction

KASSANIS and KLECZKOWSKI (1948) studied the inhibitory effect of the juice of *Phytolacca esculenta* and of glycoprotein isolated from the juice of this plant on tobacco mosaic virus (TMV). They also investigated the effect on tomato bushy stunt virus, potato virus X, tobacco necrosis virus and cucumber mosaic virus. They demonstrated that glycoprotein combines with TMV and found an immediate reduction in the infectivity of the virus in a mixture with the inhibitor. BRČÁK, POLÁK and PINTERA (1957) examined the inhibitory effect of fresh juice from *Phytolacca americana* and *P. acinosa* on infection by TMV and beet mosaic virus and obtained almost identical results. KUNTZ and WALKER (1947) found two inhibitory substances in fresh spinach juice. The properties of one of these indicate that it may have the character of a protein.

A number of proteins are already known to act as inhibitors of plant viruses. Among the most effective is ribonuclease (LORING 1942), the inhibitory activity of which can, according to KASSANIS and KLECZKOWSKI, be compared with that of the above-mentioned glycoprotein isolated from *P. esculenta*.

In continuation of previous work (POLÁK 1958), which demonstrated the inhibitory effect of saliva on TMV infection, an investigation was made of the inhibitory effect of mucin on TMV infection and of the effect of mucoprotein isolated from urine, which is known to combine with some animal viruses (GOTTSCHALK 1952, TAMM, BUGHER and HORSFALL 1955).

### Material and Methods

Both mucoproteins were isolated at the Oncological Institute, Brno, in Dr. Brada's laboratory and were kindly supplied by him for the experiments. (Mucin was isolated by BLIX's method (1936) from the submaxillary glands of cattle, urinary mucoprotein according to TAMM and HORSFALL (1950) from human urine).

Both infective juice prepared by homogenization of leaves of *Nicotiana tabacum* L., var. SAMSUN systemically invaded with the yellow strain of TMV (*Nicotiana virus* 1C), and a solution of purified green strain of TMV (*Nicotiana virus* 1) in distilled water were used for the experiments. The purified TMV was prepared by isoelectric precipitation by the method of COMMONER et al. (1950). The concentration of the original solution of this preparation was determined by testing for phosphorus content. One ml. of the preparation contained 9 mg.

of purified TMV. The solutions of both mucoproteins were made by dissolving 0.0625 g. of substance in 2 ml. of 0.05 M NaOH. About 20 ml. of distilled water was then added and the pH of the solution was adjusted to 7.0 with 0.1 N HCl. Volume was made up to 25 ml. with distilled water. The concentration of the solution then corresponded to the concentration of mucin in human saliva (INOUE 1930). The control mixture was made up by adding the same amount of 0.05 M NaOH to distilled water, adjusting the pH to 7.0 and making up to 25 ml. Determination of the effect of both substances on infectivity of TMV was carried out on half-leaves of *Nicotiana glutinosa* L. Either infective juice or a solution of purified TMV was mixed with the solution of the substance and then tested on half-leaves of *Nicotiana glutinosa*. In the second case leaves of *N. glutinosa* were rubbed with the solution of the substance and then with infective juice. The inoculum was rubbed with a camel hair brush on the leaves of already decapitated plants, which had been dusted before inoculation with 600-mesh carborundum powder. Sets of the number of lesions on left (control) and right (experimental) halves of the leaves were analyzed statistically (HRUBÝ 1950). The arithmetical average of the sets of the numbers of lesions was determined with its median error ( $\bar{x} \pm 3 s_{\bar{x}}$ ) as well as the significance of the differences of probability (P). Percentage of inhibition was obtained by means of UTECH—JOHNSON'S formula (1950).

## Results and Discussion

### A. Inhibitory effect of mucin on TMV infection

Mucin solution was tested in a mixture with infective juice in the proportion of 1 : 1 as compared with a corresponding mixture of infective juice and distilled water. The test was carried out immediately following the preparation and thorough shaking of the mixture. The result is given in Table 1a. The results of similar experiments, in which only 10 leaves of *N. glutinosa* were used for one test, fluctuated above and below the border line of the statistical significance of differences of probability and inhibition fluctuated between 20—35%. In mixtures left after shaking for 20 hours in a refrigerator (2—7° C) no marked decrease in infectivity was found.

When, however, the same experiment was carried out with a solution of purified TMV diluted with distilled water in the proportion of 1 : 1, a marked increase in the percentage of inhibition was obtained as compared to the level in the above experiments with infective juice (Table 1b). Here also the percentage inhibition was not noticeably altered by leaving the mixture of purified TMV and mucin solution in the refrigerator before testing.

In further experiments the effect of mucin solution on a solution of purified TMV was compared with that of fresh human saliva. The purified TMV, diluted with distilled water in the ratio of 1 : 5, was mixed both with mucin solution (Table 1c) and with saliva (Table 1d) in proportions of 1 : 1. A mixture of similarly diluted purified TMV with adjusted distilled water was used as the control. The results indicate that the inhibitory effects of mucin and human saliva on purified TMV, when tested immediately following mixing and thorough shaking, are practically identical. In a further test purified TMV diluted only with the same volume of distilled water (a stronger solution than in the previous experiment) was mixed with saliva in equal parts and left for 3 hours at laboratory temperature before use. The control was a mixture of similarly diluted purified TMV with adjusted distilled water in the appropriate proportions. The percentage of inhibition was increased in spite of using the stronger concentration of purified TMV (Table 1e).

Table 1

| Exp. | $\bar{x} \pm 3 \cdot s_{\bar{x}}$ |                       | n  | P <              | % inhib. |
|------|-----------------------------------|-----------------------|----|------------------|----------|
|      | control                           | test                  |    |                  |          |
| a    | 262.85 $\pm$ 3. 24.29             | 170.25 $\pm$ 3. 21.63 | 20 | 10 <sup>-2</sup> | 35.2     |
| b    | 168.60 $\pm$ 3. 24.01             | 38.95 $\pm$ 3. 7.36   | 20 | 10 <sup>-6</sup> | 76.8     |
| c    | 14.87 $\pm$ 3. 3.49               | 1.26 $\pm$ 3. 0.26    | 15 | 10 <sup>-4</sup> | 91.4     |
| d    | 14.74 $\pm$ 3. 3.15               | 1.53 $\pm$ 3. 0.51    | 15 | 10 <sup>-4</sup> | 89.5     |
| e    | 37.05 $\pm$ 3. 3.25               | 2.30 $\pm$ 3. 0.409   | 20 | 10 <sup>-9</sup> | 93.7     |
| f    | 18.80 $\pm$ 3. 2.57               | 4.00 $\pm$ 3. 0.66    | 20 | 10 <sup>-7</sup> | 78.7     |
| g    | 18.43 $\pm$ 3. 3.97               | 3.57 $\pm$ 3. 0.73    | 16 | 10 <sup>-3</sup> | 80.6     |

### B. Inhibitory effect of urinary mucoprotein

The solution of urinary mucoprotein was tested in a mixture with infective juice in the proportion of 1 : 1. The control mixture contained a corresponding amount of infective juice and distilled water. The result shows that there is a strong inhibitory effect (Table 1f).

In the next experiment the effect of urinary mucoprotein on purified TMV was examined and the same experiment was carried out in the presence of fresh juice from healthy tobacco in the mixture. For this purpose three mixtures were prepared as follows (the substances were added to the mixtures in the order given; after each addition the mixture was shaken):

A — 2 ml. purified TMV + 2 ml. distilled water + 4 ml. mucoprotein solution,

B — 2 ml. purified TMV + 2 ml. fresh healthy juice + 4 ml. mucoprotein solution,

C — 2 ml. purified TMV + 6 ml. distilled water.

The rates of infectivity of the three mixtures were mutually compared (Table 2).

An investigation was made as to whether the infectivity of the mixture of purified TMV, mucoprotein and fresh tobacco juice is dependent on the order in which the components are added. On this occasion not only fresh juice was used, but also clarified juice (heated to 65° C for 12 minutes and centrifuged at 10,000 G for 5 minutes). The following mixtures were tested:

A — 1 ml. purified TMV + 1 ml. mucoprotein + 2 ml. distilled water,

Table 2

| Mixture | $\bar{x} \pm 3 \cdot s_{\bar{x}}$ | Mixture | $\bar{x} \pm 3 \cdot s_{\bar{x}}$ | n  | P <              | % inhib. |
|---------|-----------------------------------|---------|-----------------------------------|----|------------------|----------|
| C       | 222.15 $\pm$ 3. 28.48             | A       | 1.95 $\pm$ 3. 0.38                | 20 | 10 <sup>-9</sup> | 99.1     |
| C       | 266.70 $\pm$ 3. 24.88             | B       | 32.10 $\pm$ 3. 5.60               | 20 | 10 <sup>-9</sup> | 87.9     |
| A       | 21.30 $\pm$ 3. 6.67               | B       | 78.65 $\pm$ 3. 12.46              | 20 | 10 <sup>-4</sup> | 72.9*    |

\* value indicates increase in infectivity of mixture B (containing fresh juice) compared with mixture A.

- B — 1 ml. purified TMV + 1 ml. mucoprotein + 2 ml. fresh healthy juice,  
 C — 1 ml. purified TMV + 1 ml. mucoprotein + 2 ml. clarified healthy juice,  
 D — 1 ml. mucoprotein + 2 ml. distilled water + 1 ml. purified TMV,  
 E — 1 ml. mucoprotein + 2 ml. fresh healthy juice + 1 ml. purified TMV,  
 F — 1 ml. mucoprotein + 2 ml. clarified healthy juice + 1 ml. purified TMV.

After the addition of each component the mixtures were shaken on the electric shaker for 5 minutes. The rates of infectivity of the mixtures were tested in threes, A—C and D—F. Results are given in Table 3.

Table 3

| Mixture | $\bar{x} \pm 3 \cdot s_{\bar{x}}$ | Mixture | $\bar{x} \pm 3 \cdot s_{\bar{x}}$ | n  | P <                | % increase of infectivity |
|---------|-----------------------------------|---------|-----------------------------------|----|--------------------|---------------------------|
| A       | 20.00 $\pm$ 3.1.82                | B       | 23.75 $\pm$ 3.3.38                | 20 | 5.10 <sup>-1</sup> | 15.7+                     |
| A       | 14.90 $\pm$ 3.1.53                | C       | 31.15 $\pm$ 3.4.10                | 20 | 10 <sup>-3</sup>   | 52.1                      |
| B       | 16.75 $\pm$ 3.1.76                | C       | 28.10 $\pm$ 3.3.10                | 20 | 10 <sup>-2</sup>   | 40.3                      |
| D       | 5.40 $\pm$ 3.0.61                 | E       | 13.80 $\pm$ 3.1.45                | 20 | 10 <sup>-7</sup>   | 60.8                      |
| D       | 6.75 $\pm$ 3.0.93                 | F       | 13.85 $\pm$ 3.1.67                | 20 | 10 <sup>-3</sup>   | 51.2                      |
| E       | 20.35 $\pm$ 3.2.44                | F       | 16.45 $\pm$ 3.1.90                | 20 | 3.10 <sup>-1</sup> | 19.1*                     |

\* value indicates % inhibition; difference between E and F not statistically significant. + result not statistically significant.

In order to find out whether the preparation affects the sensitivity of the host, the right halves of leaves of *N. glutinosa* were rubbed with urinary mucoprotein solution one hour before rubbing the infective juice. At the same time the left halves were rubbed with distilled water one hour before infective rubbing. A statistically significant difference in the number of local lesions was obtained (Table 1g).

The inhibitory effect of mucin and urinary mucoprotein on TMV infection is considered to have been experimentally established. The action of these substances is immediate and is clearly slowed down, in the case of mucin almost halted, by fresh tobacco juice. These findings are in complete agreement with those of KASSANIS and KLECZKOWSKI (1948), who also consider that inhibitors of the nature of proteins may combine with some of the substances of plant cells.

In studying the inhibitory effect of human saliva on TMV infection (POLÁK 1958), which formed the starting point for the present work, it was found that the low percentage of inhibition of a mixture of fresh infective juice and saliva when tested immediately following mixing is considerably increased after 16 hours storage in a refrigerator. This result is in agreement with the experiment described above, when a mixture of purified TMV and saliva showed a higher percentage of inhibition after standing for 3 hours (although the concentration of the purified TMV solution was higher than that used in the test of the immediate effect of saliva on purified TMV). On the other hand, the infectivity of a mixture of purified TMV and mucin was not noticeably

altered by standing. It was also found that rubbing half-leaves with mucin solution one hour before rubbing with infective juice (corresponding control halves rubbed with adjusted distilled water and then in an hour with infective juice) gave a negative result in all cases as opposed to the result of rubbing with saliva (POLÁK 1958). It may be concluded from the above that another inhibitor besides mucin is active in saliva and that its action is not immediate. PIRIE (1958) mentions that ribonuclease was identified in saliva and that it inactivates the nucleic acid of TMV within a few hours at 20° C even in very diluted saliva).

Urinary mucoprotein is a stronger inhibitor than mucin, achieving almost 100% effect in test with purified TMV. A detailed examination was made of the mutual relation between purified TMV, fresh juice from healthy tobacco and mucoprotein. Experiments showed that the resultant infection from mixtures of these substances depends on the order in which they are added: 1. When mucoprotein solution is added to a mixture of fresh juice and purified TMV, the inhibitory activity of mucoprotein is reduced by the presence of fresh juice. It is supposed that in this case the mucoprotein combines both with TMV and with a substance contained in fresh tobacco juice. This experiment also serves to explain the difference in the effect of mucoprotein on infection with purified TMV (almost 100% effect) and with fresh infective juice (inhibitory effect about 79%). 2. When fresh healthy tobacco juice is added to a mixture of purified TMV with mucoprotein solution it has no effect on the resultant infectivity of the mixture. In this case, then, the combination of TMV and mucoprotein is not disturbed. 3. With the same procedure as in case 2 clarified juice significantly increases the resultant infectivity of the mixture. It is probable that it binds a part of the mucoprotein which was in combination with TMV before the clarified juice was added. 4. Reduction of the inhibitory activity of mucoprotein also takes place in mixtures prepared in the order mucoprotein, fresh juice and purified TMV. In this case, however, there was no statistically significant difference between fresh and clarified juice.

An informative experiment was also carried out to determine the effect of fresh juice from healthy tobacco by itself on infection by purified TMV. The result was not statistically significant.

Urinary mucoprotein also affects the sensitivity of hypersensitive test plants to infection by TMV. This was demonstrated by rubbing half-leaves of *N. glutinosa* with a solution of mucoprotein before rubbing them with infective juice.

#### Acknowledgment

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Address: Zdenko Polák, Prom. Biol. Fytopatologické odd. Biologického ústavu, ČSAV, Praha 6-Dejvice, Na Karlovce 1.

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## Действие двух мукопротеидов на инфекцию вирусом табачной мозаики

Зденко Полак

Отдел фитопатологии Биологического института ЧСАН, Прага

### Резюме

Автор исследовал ингибиторное действие муцина, выделенного из субмаксиллярных желез коровы, и мукопротеида из человеческой мочи на инфекцию вирусом табачной мозаики (ВТМ) методом местных некрозов на половинах листьев *Nicotiana glutinosa* L. Оба препарата исследовались, с одной стороны, в смесях с инфекционным соком из растений *N. tabacum* L. (сорта Самсун, заболевших желтым штаммом ВТМ), с другой стороны, в смесях с раствором пурификата зеленого штамма ВТМ.

Ингибиторное действие муцина на инфекцию пурификатом ВТМ (в смеси обоих веществ в отношении 1 : 1) достигает 76,8 %. Однако, оно настолько снижается в присутствии сырого сока из табака, что в смеси с инфекционным соком подавление достигает лишь 20—35%.

Далее сравнивалось подавляющее действие муцина с действием свежих слюней. Из этих опытов вытекает, что слюни, кроме муцина, содержат еще другое ингибиционное начало, которое, в отличие от первого, не обладает непосредственным действием.

Мочевой мукопротеид является более эффективным ингибитором, и его действие также проявляется непосредственно. В смеси с инфекционным соком в отношении 1 : 1 его тормозящее действие достигает 78,7%. Инфекция пурификатом ВТМ (при  $P < 10^{-9}$ ) тормозится почти полностью. Из этих опытов, так же как и из результатов опытов с муцином, ясно вытекает различие в действии ингибиторов на инфекционный сок и на раствор пурификата ВТМ. Поэтому исследовалась комбинационная способность мочевого мукопротеида с пурификатом ВТМ и с сырым соком из здорового табака. Было установлено, что инфекционная способность смеси этих веществ зависит от того, в каком порядке производится их смешивание. Из опытов вытекает, что мукопротеид комбинируется как с ВТМ, так с неустановленным веществом, содержащимся в сыром соке табака.

## Zur Problematik der endogenen Virusbildung

GERHARD STAAR,

Phytopathologisches Institut der Friedrich Schiller-Universität, Jena, DDR

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### Souhrn

Autor podává zprávu o své současné práci. U viros předchází projevu zevně znatelných příznaků cytologické změny. Jako takové se popisují mimo jiné charakteristické buněčné inkluse v mladých klíčních bramborových hlíz. Jejich existenci bude možno dokázat, zda virovní procesy se mohou dít i v takových hlízách, které byly otestovány jako „zdravé“.

Nestejně silný výskyt buněčných jader vřetenovitěho tvaru v palisádovém parenchymu listů *Solanum tuberosum* lze považovat za znak různě silně vyhraněné náklonnosti k virovému onemocnění (virofilie). Pokusy indukovat virovní procesy chemickými a fyzikálními zásahy, vedly k pozoruhodnému zvýšení výskytu vřetenovitých jader v takto ošetřovaných rostlinách.

Virogenesi považujeme v podstatě za fyziologický proces, řízený rostlinou a vyvíjející se jak post infectionem tak i autonomně. Výskyt virogenních mikroprocesů i virovních mikroohnisek dokazatelných anatomicky znemožňuje důkaz, že nějaká rostlina je viru prostá. Spíše lze mít za to, že tento výskyt je ubiquitní.

### Zusammenfassung

Es wird über laufende Arbeiten berichtet. — Bei virösen Erkrankungen gehen dem Auftreten äusserlich erkennbarer Symptome cytologische Veränderungen voraus. Als solche werden u. a. charakteristische Zelleinschlüsse in jungen Keimen von Kartoffelknollen beschrieben. Mit ihrer Hilfe lässt sich nachweisen, dass viröse Prozesse auch in „gesund“ getesteten Knollen vorkommen können. — Das unterschiedliche Auftreten spindelförmiger Zellkerne im Palisadenparenchym der Blätter von *Solanum tuberosum* wird als Zeichen verschieden stark ausgeprägter Neigung zu viröser Erkrankung (Virophilie) angesehen. Versuche zur chemisch-physikalischen Induktion viröser Prozesse ergaben ein bemerkenswertes Auftreten derartiger Kernformen in behandelten

Pflanzen. — Die Virogenese wird als ein im Wesentlichen physiologischer Prozess aufgefasst, den die Pflanze gestaltet und der sich sowohl post infectionem als auch autonom entwickeln kann. Das anatomisch nachweisbare Vorkommen virogener Mikroprozesse und viröser Mikroherde macht den Nachweis, dass eine Pflanze virusfrei ist, unmöglich. Vielmehr ist mit der Ubiquität dieses Vorkommens zu rechnen.

### Einleitung

Neben der meist vertretenen Auffassung, dass viröse Prozesse in einem Organismus nur nach vorangegangener Importation von Virus in Gang kommen bzw. ablaufen können, findet man Angaben über die Existenz von „latenten“ und „apathogenen“ Viren. Auch von „Proviren“, „nichtinfektiösen Vorstufen“ u. a. und der Möglichkeit ihres Übergehens in pathogene Formen ist die Rede. Hier können nur einige und besonders charakteristisch scheinende Äusserungen herangezogen werden.

Als unmittelbarer Vorläufer der Theorie der endogenen Virusbildung war HUNGER (1905) der Ansicht, dass bestimmte in den Zellen der Tabakpflanze produzierte Substanzen eines übersteigerten Stoffwechsels bei Anhäufung und Überkonzentrierung pathogen werden können. Von Virus in unserem Sinne ist bei ihm allerdings noch nicht die Rede.

SCHAFFNIT (1927) hielt eine Entstehung von Virus als Eiweissgift in der Pflanzenzelle unter besonderen äusseren und inneren Bedingungen für denkbar.

REILING (1929) warf die Frage auf, ob nicht ungünstige Umweltbedingungen zu Veränderungen der Eiweissubstanzen in der Pflanze Anlass geben, die schliesslich zur Entstehung eines Giftes, des Virus, führen. Ähnlich lautet die Formulierung bei FUCHS (1938).

In einer Studie über den Kartoffelabbau unter Berücksichtigung der Blattrollkrankheit sagt WARTENBERG (1949): „Das offenbar innige Einfügen des Erregers in das Getriebe der Pflanze und die Tatsache, dass auch andere, nichtinfektiöse Ursachen ähnliche Symptome hervorrufen können, verwischen den Gegensatz von Erreger und erkrankender Substanz. Was liegt näher, als das nichtinfektiöse Entstehen einer erkrankten Substanz zu vermuten, welche autokatalytisch einen sonst langsam verlaufenden chemischen Vorgang durch ihr blosses Vorhandensein beschleunigend, die Bildung von ihresgleichen anzuregen vermag und sich infektiös übertragen lässt“ (l. c. S. 140). Zugleich werden allerdings die sehr erheblichen Schwierigkeiten des Nachweises einer solchen Neuentstehung von Virus betont.

SCHWEZOWA (1950) berichtete über die künstliche Induktion der Polyederkrankheit bei Raupen der Bienenmotte durch Verfüttern kohlenstoffreicher Nahrung.

Über die Möglichkeit einer Umwandlung nichtinfektiöser Eiweisstoffe in Viren, die Existenz von apathogenen Viren, Proviren oder nichtinfektiösen Virussubstanzen äusserten sich u. a. BAWDEN u. PIRIE (zit. n. BLUNCK 1955, S. 329), BANFIELD u. Mitarb. (1951), EDLINGER (1957), JACOB (1954), JOHNSON (1942), RYSCHKOV (1955), STANLEY (1957) und WEIDEL (1957).

THUNG (1957) betont -übrigens ganz im Sinne der von uns vertretenen Auffassung- die Rolle der betroffenen Pflanze bei der Virogenese. Wenn ein Virus in einer bestimmten Pflanzenart pathogen ist, in einer anderen aber nicht, so entscheidet die Pflanze über die Funktion des betr. Agens, m. a. W. darüber, ob es ein Virus ist oder was sonst. In letzter Konsequenz sieht THUNG die Möglichkeit, dass aus einer normalen Pflanzen-Nukleinsäure unter dem Einfluss bestimmter Faktoren ein Agens mit Virusfunktion oder ein Vollvirus hervorgeht.

Aus dem Bemühen, zwei Anschauungen, die ökologische und die virologische Kartoffelabbau-Theorie zu kombinieren, wurde um die Zeit nach 1930 im Institut für Pflanzenkrankheiten zu Landsberg Warthe die Hypothese der endogenen Virusbildung entwickelt. Sie besagte, dass fortgesetzter ökologischer Abbau der Kartoffel von progressiven Funktionsstörungen begleitet ist, die von einem gewissen Stadium ab irreversibel werden und schliesslich zur Produktion von Virus führen können, wobei die autokatalytischen Prozesse im Stoffwechsel der Zelle eine wesentliche Rolle spielen.

Wir begannen mit der anatomischen Untersuchung von Geweben und Zellen abbaukranker Kartoffelpflanzen auf das Vorhandensein charakteristischer „innerer Symptome“ und stiessen auf Gewebeschwund (Hypotrophie), Unregelmässigkeiten in der Kalziumoxalat-Verteilung (Vakuenen), Zellkerndeformationen, Chloroplastendefekte, x-bodies u. a. m. In den Jahresberichten des o. g. Instituts von 1929 bis 1934 erschienen kurze Mitteilungen darüber. Mit SCHANDER's Tod sind diese Arbeiten zum Stillstand gekommen.

Erst ca. 20 Jahre später ergab sich die Möglichkeit, das seinerzeit Begonnene fortzusetzen und durch die Aufnahme von Versuchen zur künstlichen Induktion der Virusbildung ohne Importation von Vollvirus auszubauen. Zugleich ergab sich die Notwendigkeit einer Angleichung unserer Arbeitshypothese an den heutigen Stand der Virusforschung. Die Hauptlast der experimentellen Arbeiten trug die Dipl. Biologin Frä. Lilli Vogler, der ich an dieser Stelle dafür danken möchte.

Wer sich prinzipiell für die Auffassung der Viren als Organismen entscheidet, darf die Frage einer möglichen endogenen Virusbildung nicht ernstlich erwägen, denn er müsste mit der *generatio spontanea* s. str. rechnen. Angesichts der komplizierten Struktur der Viren wäre das nicht vertretbar. Diskutierbar dagegen ist u. E. die Möglichkeit einer stufenweisen Synthese von Eiweisskörpern mit Viruscharakter aus dem pflanzlichen Stoffwechsel selbst heraus. Eine solche Endogenese müsste natürlich irgendwann und irgendwo beginnen. Wir werden uns damit abfinden müssen, dass wir dieses Ereignis nicht beobachten können. Die Massnahmen zur Beobachtung müssen sozusagen am Nullpunkt, also bei irgendeiner mehr oder weniger willkürlich für „normal“ gehaltenen (oder erklärten) Zelle einsetzen, und es bliebe abzuwarten, ob es der Zufall will, dass gerade in dieser Zelle der gewünschte Prozess anläuft. In jedem Falle hatte der Beobachtungsvorgang irgendeinen unkontrollierbaren Einfluss auf das Geschehen im Beobachtungsobjekt, so dass das Beobachtungsergebnis nicht eindeutig auf eine bestimmte Ursache zurückgeführt werden könnte.

Wir gehen deshalb den umgekehrten Weg und suchen das virale Geschehen zunächst anhand zellanatomischer Befunde rückwärts zu verfolgen, um

möglichst viele Entwicklungsstadien kennen zu lernen und daraus den Entwicklungsverlauf zu rekonstruieren. Dabei sind wir uns der Gegensätzlichkeit unserer Auffassung zu DOERR (1944, S. 25) durchaus bewusst. Die Schlussfolgerungen können selbstverständlich nur den Wert eines Indizienbeweises beanspruchen. Wir greifen zunächst auf die jüngsten Entwicklungsstadien der Kartoffelpflanze zurück, die Keime der Knolle. Später sollen auch die Samenkeime und die jungen Keimpflanzen in die Untersuchungen einbezogen werden.

Die bei diesen Studien als geeignet erkannten cytologischen Merkmale sollen dann Aufschluss darüber geben, ob auch in sogen. „gesunden“, nicht als viruskrank diagnostizierbaren Pflanzen viröse Mikroprozesse vorkommen. Sie dienen ferner zur Beurteilung des Effektes bei den Versuchen zur künstlichen Induktion der endogenen Virusbildung.

## Materiale, Methoden und Ergebnisse

### I.

Bei früheren Untersuchungen an mosaikkranken Blättern der Kartoffel war das fleckweise Fehlen der in gesunden Blättern ziemlich gleichmässig verteilten Kalziumoxalatzellen aufgefallen und als diagnostisches Merkmal für Mosaik-Virosen empfohlen worden (Jahr. Ber. Landsbg. 1928). Mein Mitarbeiter Dr. Vorsatz hat nachgewiesen, dass diese sogen. Oxalatvakanzen nicht zu den Frühsymptomen gehören, sondern erst in einem späteren Stadium der virösen Erkrankung und zwar nach Erscheinen der Mosaikfleckung auftreten. In den Blättern äusserlich gesunder Kartoffelpflanzen sind die Kristallsandzellen zwar verschieden dicht, aber im allgemeinen recht gleichmässig verteilt. Ein Teil von ihnen ist nicht vollständig gefüllt und in solchen Zellen der Inhalt häufig mehr oder weniger deutlich korrodiert. Derartige Abweichungen treten anscheinend regellos, aber besonders oft bei Lichtmangel auf (VORSATZ 1957). Gelegentlich werden Blätter gefunden, in denen die Kristallsandzellen innerhalb unscharf begrenzter Bezirke weniger dicht gelagert sind (Abb. 1). Manches spricht dafür, dass es sich hier, also bei „gesunden“ Blättern, um die ersten Stadien der Bildung von Kalziumoxalatvakanzen handelt.

Seinerzeit (Jahresber. Landsbg. 1930) waren auch Kerndeformationen in den Palisadenzellen insbesondere rollkranker Blätter und solcher gefunden worden, die die äusseren Symptome des Aukubamosaiks zeigten. Der Zellkern in den Palisadenzellen „gesunder“ Blätter der Kartoffel ist nach unseren bisherigen Beobachtungen rund bis stumpf-oval. In rollkranken Blättern sind alle Übergänge von der einseitigen Zuspitzung über die typische Tropfenform bis zur beiderseitig geisselförmig ausgezogenen Spindel anzutreffen (Abb. 2). Die spindelförmigen Zellkerne in aukubabunten Blättern sind grösser als die in rollkranken, ein Faktum, das uns schon seinerzeit veranlasst hatte, das sogen. „Aukubabunt“ ätiologisch aus der Gruppe der Mosaikkrankheiten herauszunehmen. Gelegentlich und zwar ebenfalls in den Palisadenzellen wurde die Nukleolenverdopplung und -fragmentierung sowie das Auftreten extranuklearer Nukleolen beobachtet, bei denen es sich übrigens nicht um mikrotechnische Artefakte gehandelt hat.

Von den früheren Befunden, die sich neuerdings bestätigten, ausgehend suchten wir jetzt Aufschluss darüber zu gewinnen, ob eine „Kernformen-Statistik“ zur Charakterisierung viröser Prozesse geeignet ist resp. ob die Entwicklung von Spindelkernen in einem Zusammenhang mit der Entwicklung oder dem Ablauf viröser Prozesse steht.

Die für diesen Zweck notwendige reine Kernfärbung ohne gleichzeitige Anfärbung des übrigen Zellinhalts, insbesondere der Plastiden, erreichten wir bisher nur mit Hilfe der Feulgen-Methode. Lediglich bei rollkranken und bei den albikaten Partien mosaikkranker Blätter haben wir den gewünschten Färbefekt z. B. auch mit Kresylechtviolett und mit Eisenhämatoxylin erhalten. Hier werden die offenbar denaturierten Plastiden nicht mitgefärbt bzw. beim Differenzieren wieder entfärbt.

Form und Grösse der Kerne in den Palisadenzellen erwiesen sich als recht variabel. Die Intensität der Feulgen-Färbung verhält sich zur Längsausdehnung der Zellkerne in den Palisadenzellen anscheinend umgekehrt proportional.

Die Kernformen-Statistik gestattet keine direkten Schlüsse auf den Beginn oder den Verlauf viröser Prozesse und deren spezifischen Charakter, sondern sie spiegelt lediglich die mehr oder minder ausgeprägte „Virophilie“ einer Kartoffelsorte wieder. Wir verstehen unter Virophilie die Neigung der betr. Sorte oder auch einer einzelnen Pflanze zu virogenen und virösen Prozessen, m. a. W. ihre normale Prädisposition für diese. So fanden wir bei der stark unter Abbauerscheinungen leidenden Sorte Ackersegen auch in äusserlich gesunden Pflanzen überwiegend Spindelkerne und daneben einige Tropfenformen, bei der im Feldbestand rel. weniger abbaufähigen Sorte Aquila hingegen überwiegend runde Kernformen.

Bei den früheren Untersuchungen an alten, längst verschwundenen Sorten war die gleiche Tendenz zutage getreten. Man sollte daher die Bedeutung der Kernformen-Statistik für die Züchtung abbauresistenter Kartoffelsorten prüfen. Als leistungsfähige Routinemethode schwebt uns die Auszählung von Zellkernsuspensionen vor, die aus dem Homogenisat fixierter Blätter gewonnen werden. Der in derartige Suspensionen mit eingehende Anteil an Zellkernen aus dem Schwammparenchym dürfte sich dabei anhand morphologischer Merkmale weitgehend eliminieren lassen. Entscheidend ist stets nur die relative Anzahl der Spindel- und Tropfenformen.

## II.

Bei der Suche nach den Anzeichen für viröse Prozesse in den Zellen der Keime von Kartoffelknollen stiessen wir auf eigentümliche Einschlusskörper (Inklusen), die sich von den x-bodies wesentlich unterscheiden.

ROSENZOFF (1949) hat eine umfangreiche Zusammenstellung von Einschlusskörpern in den Zellen gegeben und konnte selbst die Virusnatur der in einigen Kakteen beobachteten Eiweiss-spindeln wahrscheinlich machen, als es ihr gelang, die Bildung spindelförmiger Inklusen in bis dahin spindelfreien Pflanzen durch Pfropfung, Impfung und Transplantation zu induzieren. Immerhin blieb auch noch die Deutung als Nebenprodukte nichtviröser Art in dem Stoffwechsel virös erkrankter Zellen möglich.

Den Beweis für die viröse Natur dieser Inklusen erbrachte AMELUNXEN (1956, 1958). Er konnte bei verschiedenen *Opuntia*-Arten die Bildung der Spindeln aus x-bodies als Parallel-aggregation der in diesen vorhandenen Partikeln verfolgen. Schliesslich gelang es ihm, die lichtmikroskopisch erkennbaren Fibrillen mit Hilfe des Elektronenmikroskops in einzelne Fäden aufzulösen, die ihrerseits aus einzelnen Partikeln bestimmter Länge bestanden.

Auch in Solanaceen sind spindel-, nadel- und fadenförmige Inklusen beobachtet worden. LIKHITÉ (1929) fand sie in mosaikkranken Tabakpflanzen und beschreibt sie als mehr oder weniger gerieft, m. a. W. vermutlich von ebenfalls fibrillärer Struktur. Mit Wahrscheinlichkeit kann angenommen werden, dass in Kartoffelpflanzen auftretende Inklusen dieser Art nicht anders zu beurteilen sind.

KLEBAHN (1928) beschrieb sie beim Crinkle-Mosaik, Interkostal-Mosaik, beim gewöhnlichen und beim Aucuba-Mosaik der Kartoffel: Korkenzieherartig gewundene sogen. Trypanoplasten im Phloem 5 bis 10 mm grosser Lichtkeime an Knollen von crinkle-mosaik-kranken Pflanzen neben einfacher gestalteten sogen. Ooplasten. Langgestreckte, meist schwach gekrümmte Spindeln fand er in mosaikkranken Knollen, allerdings nur sehr spärlich und vor allem im Phloem der Keime. In einzelnen Fällen „erschieden die Spindeln wie aus . . . schraubig umeinander gewundenen Fäden zusammengesetzt, und sie spalteten dann am Ende in einige dünne Fäden auf“ (l. c. S. 69/70).

CLINCH (1930) beschrieb schlanke, lange, meist homogene Körper kristalloider Natur in der Epidermis und den subepidermalen Zellschichten der Blätter von schwer an Crinkle und Intervainalmosaik erkrankten Kartoffelpflanzen, jedoch nur bei fortgeschrittenen Stadien der Krankheit.

Auf die „klassischen“ X-bodies soll in diesem Zusammenhang nicht eingegangen werden.

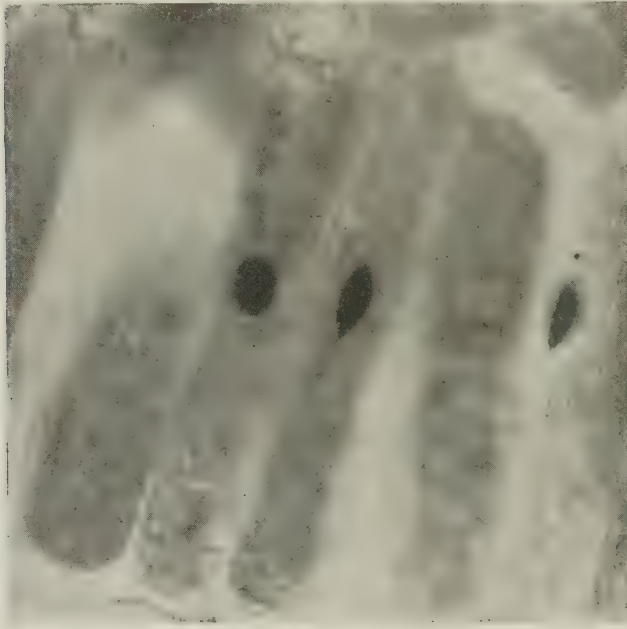
Bei unseren eigenen Untersuchungen an Licht- und Dunkelkeimen von Kartoffelknollen fanden wir fast alle in der Literatur beschriebenen Inklusenformen wieder.

Die Spindeln können fadenförmig sein oder einen stärkeren Durchmesser haben. Sie können geradegestreckt, sichelförmig gebogen oder peitschenschwurähnlich geschwungen sein und verfügen sich entweder nach beiden Enden hin oder sind einseitig abgestumpft, bisweilen auch aufgefaser (Abb. 3a,b). Derartige, von uns als „typisch“ bezeichnete Körper sind scharf



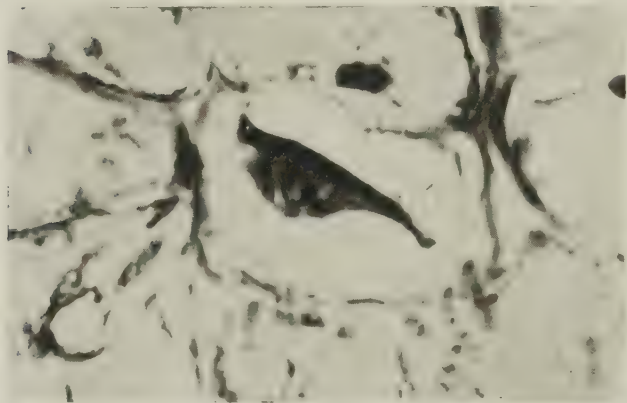
Phot. Vorsatz

Abb. 1. Äusserlich gesundes Blatt von Sol. tub. mit lokalem Kalziumoxalat-Schwund (Extraktion mit heissem Alkohol und Aufhellung in gesättigter Lösung von Chloralhydrat in Glycerin) Orig. Vergr.  $10\times$  Dunkelfeld



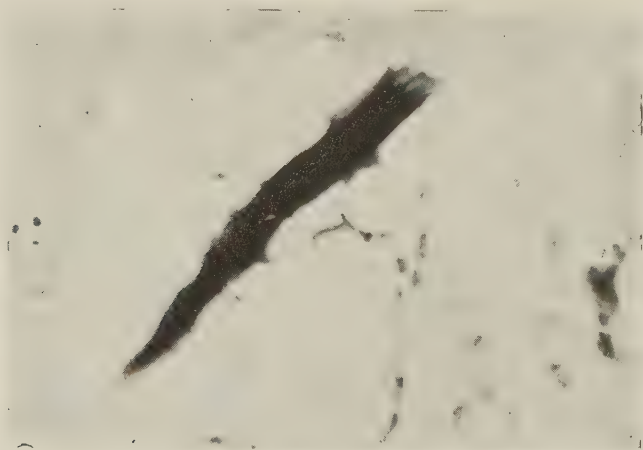
Phot. Bamberger

Abb. 2. Palisadenzellen eines Blattes von *Sol. tub.* mit einem normalen (links) und zwei Spindelkernen (rechts). Orig. Vergr. 400 $\times$ . Fixiert nach Carnoy, gefärbt Säurefuchsin



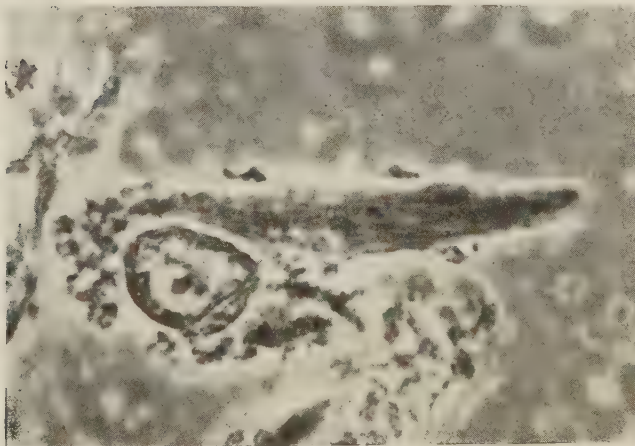
Phot. Vogler

Abb. 3a. Typische, spindelförmige Inkluse; an einem Ende abgeschrägt; am Kern anliegend und mit ihm durch Plasmamassen verbunden. Orig. Vergr. 400 $\times$ . Fixiert nach Zenker, gefärbt Hämatoxylin u. Heidenhain-Gurr



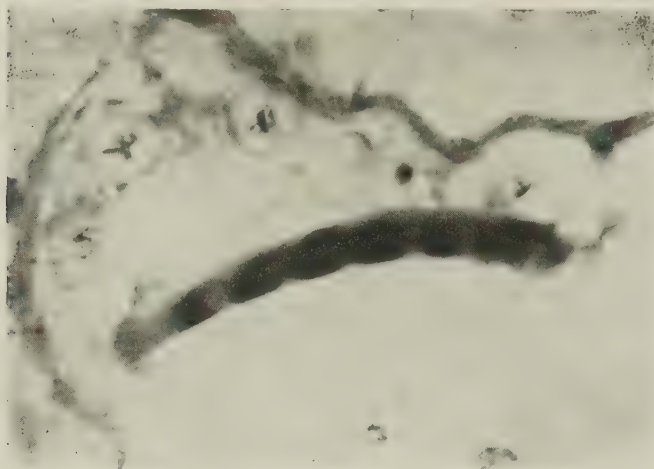
Phot. Vogler

Abb. 3b. Typische, spindelförmige Inkluse; an einem Ende aufgefasert. Orig. Vergr.  $600\times$ . Fixiert nach Zenker, gefärbt Hämatoxylin u. Heidenhain-Gurr



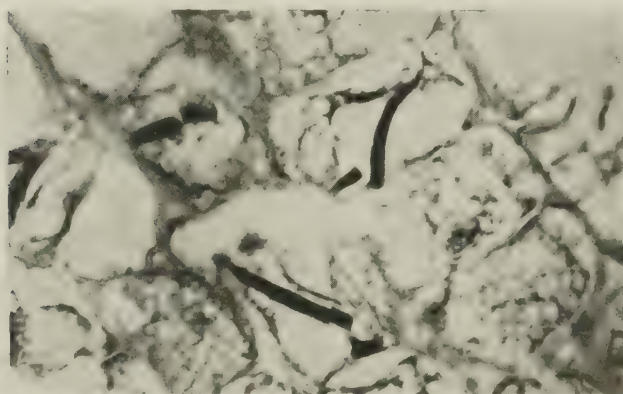
Phot. Vogler

Abb. 4. Fibrilläre Inkluse (grauer Körper) aus einem Dunkelkeim. Orig. Vergr.  $900\times$ ; Phasenkontrast. Fixiert nach Zenker, ungefärbt



Phot. Vogler

Abb. 5. „Zebrakörper“ aus einem Lichtkeim. Orig. Vergr. 900 $\times$ . Fixiert nach Zenker. Gefärbt n. Heidenhain-Gurr



Phot. Vogler

Abb. 6. Frakturen an Spindeln und Stäbchen. Orig. Vergr. 270 $\times$ . Fixiert nach Zenker. Gefärbt n. Heidenhain-Gurr

konturiert und erscheinen homogen. Sie färben sich nach Carnoy-, nicht nach Zenker-Fixierung mit Säuerfuchsin nach der von Zimmermann zur Färbung von Proteinkristalloiden angegebenen Methode brillantrot, nach Zenker-Fixierung mit Hämatoxylin nach Heidenhain in der Variante nach Gurr (Differenzierung mit Pikrinsäure und Gegenfärbung mit Safran) tief blauschwarz bis reinschwarz.

Die stäbchenförmigen Inkluden sind verschieden gross ( $20-70 \times 2-5 \mu$ ), überwiegend kurz und gedungen; ihre Längsseiten sind geradlinig und ihre Enden laufen nie in eine Spitze aus. Im Extrem wurden Stäbchen gefunden, die in ihren Länge einer Diagonale der betr. Zelle entsprachen. Neben geraden Balken kommen leicht gebogene Keilformen vor.

Sehr häufig liegen diese Gebilde in der Nähe des Zellkerns, mit diesem durch Plasmamassen verbunden. Im Gegensatz zu ihren Vorstufen sind diese vollausgebildeten Inkluden nicht immer von einer Plasmahülle umgeben.

Neben diesen typischen Spindel- und Stäbchenformen treten sehr häufig und meist gleichzeitig mit ihnen Inkluden auf, die nach der Heidenhain-Färbung nur eine violettgraue Tönung zeigen, der Form nach aber zu den Spindeln und Stäbchen gehören. Sie haben keine homogene sondern deutlich fibrilläre Struktur, erkennbar an der mehr oder weniger feinen Längsstreifung (Abb. 4). Ganz offensichtlich liegen die etwas intensiver gefärbten Fibrillen in einer schwach gefärbten Zwischensubstanz. Sie sind auch keineswegs immer parallel gelagert. Gelegentlich beobachteten wir eine zopfartige oder unregelmässige Verflechtung, wie sie REITER (1956) für Kakteen und THALER (1956) für *Lilium tigrinum* beschrieben haben. Es ist unwahrscheinlich, dass derartige Strukturen als Artefakte der Fixierung auftreten. Sie sind, wenn auch schwieriger, auch in Lebendpräparaten erkennbar. Wir halten diese „grauen Körper“ für frühe Stadien der Inkludenentwicklung.

Im Phloem der Keime traten hin und wieder auch die von KLEBAHN beschriebenen Trypanoplasten auf. In einigen Fällen wurden würfelförmige Gebilde gefunden, die den von COHN (1860), JANISCH (1942) und KOHLER (1942) für Kartoffelknollen beschriebenen Würfelkristalloiden entsprechen dürften.

Oft sind die fibrillären Inkluden mit einem kugligen Gebilde fest verbunden, das den Eindruck einer Plasmaballung mit feinkörnigem Inhalt macht. Die Ähnlichkeit mit den bekannten X-bodies ist nicht von der Hand zu weisen. Wir fanden solche Ballungen nicht nur in Keimen von Knollen mit X-virus sondern auch in einer Knolle mit S-Virus, die von einer latent kranken Pflanze stammte.

Die sogenannten Zebrakörper fanden wir nur selten: Nur in 5 von 20 Keimen und auch in diesen nur in je 1 bis 2 Exemplaren. Es handelt sich um leicht gebogene Stäbe, die nach Heidenhain-Färbung deutliche Querstreifung zeigen (Abb. 5). WEBER und KENDA (1952) berichten über analoge Beobachtungen an *Opuntia subulata*.

Häufig fanden wir zerbrochene Spindeln und Stäbe. Die Zusammengehörigkeit der Bruchstücke war fast immer deutlich erkennbar, sei es an der korrespondierenden Form der Bruchränder, sei es an den sich ergänzenden Konturen (Abb. 6). Wichtig erscheint uns die Beobachtung der Bruchlinie eines Zebrakörpers, die genau der Abgrenzung der Querbänderung folgte. Man hat den Eindruck, dass solche Bruchstellen in der inneren Struktur der betr. Inkluse irgendwie präformiert sind.

Es liegt nahe, derartige Brüche zunächst einmal als Artefakte zu betrachten; doch lassen manche Einzelheiten auch eine andere Deutung zu. Das häufige Vorkommen der Frakturen lässt darauf schliessen, dass die Konsistenz der Inkluden eher als starr oder spröde, denn als flexibel zu bezeichnen ist. Die Entscheidung muss der im Augenblick noch ausstehenden Untersuchung an lebenden Zellen vorbehalten bleiben.

Die hier beschriebenen Inkluden fanden wir in den epidermalen und subepidermalen Zellen der knollig verdickten Keimbasis und in den Zellen der ältesten Blattprimordien. Auch in den ältesten Blattanlagen von Niederknospen kommen sie vor und selbst in den Wurzelanlagen, seltener jedoch in der Nähe der Leitbündel und in den Markzellen der Keimbasis, ganz vereinzelt dann noch in den Parenchymzellen am Grunde der Keimbasis. Leitgewebe und meristematische Zellen fanden wir stets frei von Inkluden.

Gewöhnlich liegen die spindel- und stäbchenförmigen Einschlusskörper in kleineren oder grösseren unregelmässig umgrenzten Zellgruppen, die man in mehreren aufeinanderfolgenden Keimlängsschnitten immer leicht wiederfindet (vgl. KLEBAHN l. c. S. 72). Im Zusammenhang mit der Frage des Beginns der endogenen Virusbildung glauben wir diesem Verteilungsmodus eine besondere Bedeutung beimessen zu sollen.

Die kontinuierliche unmittelbare Beobachtung des Entstehens und der weiteren Entwicklung der Inkluden ist vorläufig nicht möglich. Das uns zur Verfügung stehende Material gestattet

folgende hypothetische Darstellung: Die ersten Anzeichen beginnender Inklusionsbildung sehen wir in fädigen Proliferationen der Plasmaumhüllung des Zellkerns, die anscheinend an dessen Oberfläche ansetzen und die sich dann tangential weiterentwickeln, ohne dass dabei zunächst eine Innenstruktur deutlich erkennbar wird. Je nach Lage zur optischen Achse erscheinen diese Plasmabildungen strang- oder bandförmig, wenn es sich um Plasmabänder handelt. Allmählich bildet sich dann die fibrilläre Struktur heraus. Sie ist naturgemäss in den Plasmabändern leichter und deshalb scheinbar früher zu sehen, als in den echten und den Pseudosträngen. Während die fibrilläre Struktur deutlicher wird, beginnt sich die Spindel- bzw. die Stabform immer klarer abzuzeichnen: Es formieren sich die zuvor beschriebenen „grauen Körper“. Die endgültige, „typische“ Form scheint sich dann infolge eines Zusammenrückens der Fibrillen, vielleicht unter gleichzeitigem Schwinden der Zwischensubstanz zu entwickeln. Die „grauen Körper“ verdichten sich und bekommen ausgesprochen harte Konturen, während die fibrilläre Struktur immer undeutlicher wird. Damit ist die Inklusionsbildung allem Anschein nach abgeschlossen.

Alle hier beschriebenen Entwicklungsstadien sind unabhängig von einander zu beobachten. Ihre Aufeinanderfolge in der angegebenen Reihe scheint uns der Wirklichkeit zumindest nahe zu kommen.

Ungeklärt bleibt einstweilen, ob die „Zebrakörper“ in diesem Entwicklungsschema als Abnormitäten, gleichsam als pathologische Bildungen auftreten oder ob sie Zwischenstadien einer Entwicklung mit anderem Endergebnis darstellen.

Die Eiweisnatur der von uns für die Kartoffelkeime beschriebenen Inklusionen dürfte, nach ihrem färbischen und reaktiven Verhalten zu urteilen, kaum zu bezweifeln sein. Ihre Bedeutung im virösen Prozess der Kartoffelpflanze schlechthin scheint uns der von anderer Seite für analoge Bildungen bei Kakteen angenommenen gleichzukommen, so dass wir die Inklusionen und ihre Entwicklungsstufen als charakteristische Merkmale für die Gegenwart viröser Prozesse im allgemeinen betrachten dürfen.

Theoretisch würde sich nun die Möglichkeit ergeben, mikroskopisch auch solche virösen Prozesse zu erkennen, die ihr infektiöses Endstadium noch nicht erreicht haben oder deren Gegenwart infolge ihrer geringen Ausdehnung (Mikroherde) oder Intensität weder symptomatologisch noch serologisch oder durch Übertragung nachgewiesen werden kann. Es ergibt sich weiter die Möglichkeit, ihre Entwicklung im Keimstadium der Kartoffel in Abhängigkeit von Umweltfaktoren zu untersuchen und schliesslich zu untersuchen, ob und in welchem Umfang viröse Prozesse, insbesondere deren Frühstadien, auch in „gesunden“ Pflanzen anzutreffen sind. Einige orientierende Bemerkungen seien dazu vielleicht erlaubt und geeignet, zu weiteren Arbeiten in dieser Richtung anzuregen.

In diesem Zusammenhang scheint mir die Versuchsreihe III besonders interessant zu sein. Das Knollenmaterial (Sorte Aquila und Capella) ist ausser in der Stecklingsprobe noch serologisch auf X- und S-Virus und durch Abreibungen von den Kontrollpflanzen auf A- und Y-Virus getestet worden. Ausserdem wurde in Abständen von 2 Wochen zweimal der Kallose-Test ausgeführt. Selbst unter diesen Voraussetzungen rechnen wir mit der Möglichkeit, dass die als „gesund“ getesteten Knollen inaktive Vorstufen oder aktives Virus in unerschwerlichen Mengen enthielten, die mit den üblichen Methoden noch nicht nachweisbar waren.

Sämtliche 70 untersuchten Keime wurden in lückenlose Längsschnittserien zerlegt und ebenso lückenlos durchmustert. Die Untersuchung der 25491 Einzelschnitte ergab, dass die typischen Inklusionen bei weitem häufiger in Keimen kranker als in Keimen „gesunder“ Knollen vorkamen und dass sie nicht in allen oder zumindest doch nicht gleichzeitig in allen Keimen der als krank diagnostizierten Knollen aufgetreten sind. Ihr Fehlen in einem Keim schliesst offenbar nicht aus, dass viröse Prozesse dennoch in anderen Teilen der Knolle und möglicherweise zu einem anderen Zeitpunkt ablaufen.

Besondere Beachtung verdienen zwei Keime der Kategorie „gesund“, in denen Inklusionen gefunden wurden. Es handelt sich um einen Lichtkeim von Aquila mit sehr wenigen typischen Spindeln und Stäbchen und ohne fibrilläre Strukturen, sowie einen Dunkelkeim von Capella mit sehr wenigen typischen Inklusionen, dafür aber einer grösseren Anzahl fibrillärer Körper. Es ist zu schliessen, dass in diesen beiden Fällen die als „gesund“ getesteten Knollen dennoch nicht virusfrei waren. Die vorhandene geringe Anzahl von Inklusionen lässt weiter annehmen, dass auch diese an sich virushaltigen Keime infolge unerschwerlicher Virusmengen zwei „gesunde“ Stecklinge gebracht hätten.

Für die praktische Beurteilung des Gesundheitszustandes von Kartoffelknollen — für die grünen Pflanzen gilt das gleiche — ergibt sich die Konsequenz, dass es nicht angeht, nach negativem Testbefund anhand der dabei gebräuchlichen Methoden die Diagnose „virusfrei“ zu stellen.

Im Verlauf unserer Arbeiten hatte sich bald gezeigt, dass Keime unter 3 mm Länge niemals Inklusen enthielten. Es ergaben sich aber auch keine Korrelationen zwischen dem Inklusenbesatz und der Keimlänge, gleichlange Keime können verschieden lang sein und vice versa. Die Altersunterschiede können auch bei gleich grossen Keimen sehr beträchtlich sein.

Nach unseren bisherigen Erfahrungen sind bei gleich grossen Keimen in den älteren mehr Inklusen anzutreffen als in jüngeren. Etwa 40 Tage nach Beginn der Keimentwicklung scheint die Zahl der Inklusen sprunghaft zuzunehmen.

In den Folgekeimen, die sich nach dem Absetzen des (mittleren) Hauptkeimes oder der beiden Seitenkeime an deren Stelle entwickeln, fanden wir mehr Inklusen als in den primären Keimen. Die Erstkeime können sogar inklusenfrei, ihre Folgekeime jedoch mit Inklusen besetzt sein. Eine definitive Erklärung für diesen der praktischen Erfahrung widersprechenden Befund abgekeimte Knollen sollen nach Einigen angeblich Bestände mit rel. geringerem Besatz an viruskranken Pflanzen liefern — kann vorläufig nicht gegeben werden. Möglicherweise spielen hierbei die Vegetations-, insbesondere die Lichtverhältnisse eine Rolle, denn die Folgekeime sind in dieser Beziehung zumindest in der Zeit nach Beendigung der natürlichen Winterruhe günstiger gestellt. Vielleicht ist es auch so, dass nicht das individuelle Alter des Folgekeimes, sondern der Entwicklungszustand der betr. ganzen Keimanlage (Auge) entscheidet. Wir stützen uns auf die einschlägige Literatur, nach welcher die Inklusenbildung bei *Valerianella* im Sommer früher als im Winter einsetzt und bei *Rumex obtusifolius* die X-bodies im Herbst und im Winter seltener zu finden sind als im Frühjahr. Bei der Kartoffel kommt die natürliche physiologische Ruheperiode hinzu. Es ist denkbar, dass die Inklusenbildung erst nach deren Abklingen beginnt und mit wachsender Intensität der physiologischen Prozesse zunimmt.

Bei Dunkelkeimen sind die Inklusen weniger häufig anzutreffen und allem Anschein nach sind die fibrillären (Vor-) Stadien vorherrschend. Daraus könnte man auf eine Verlangsamung der virösen Prozesse in Dunkelkeimen schliessen. Der Beweis wird sich führen lassen, wenn die Keime einer verschiedenen, hinsichtlich Dauer und Intensität genau definierten Lichteinwirkung ausgesetzt werden. Bei dieser Gelegenheit wird es zweckmässig sein, das Verhalten der typischen Inklusen während der weiteren Entwicklung der Keime zu Jungpflanzen zu verfolgen.

### III.

Den letzten Teil unserer Arbeiten bilden die Versuche zur Induktion der Virusbildung unter Ausschluss jeglicher Infektion. Zu diesem Zweck schaffen wir, unserer Arbeitshypothese entsprechend, Wachstumsbedingungen, unter denen die Fehlsynthese im Eiweisstoffwechsel begünstigt wird. Die auf Grund sorgfältiger Testung als „gesund“ ausgewählten Knollen werden halbiert und in 16 cm — Tontöpfe ausgelegt. Die Füllung besteht aus gedämpfter Komposterde, eine Torfmuldecke setzt die Wasserverluste herab. Das Giesswasser wird den Töpfen von unten her zugeführt. Kurz nach dem Auflaufen werden die Jungpflanzen in die Lichtkammer gebracht, wo sie bis zum Absterben des Krautes im Dauerlicht von Leuchtröhren der Type T (Tageslicht) stehen. Die Beleuchtungsstärke wurde mit 3068 bzw. 4238 Lux (im Abstand von 50 bzw. 25 cm) gemessen. Die Raumtemperatur wird auf 15 bis 18° C gehalten, um das Vergeilen so weit wie möglich zu unterdrücken und um den Knollenansatz zu begünstigen. Während des Wachstums werden die Pflanzen in bestimmten Abständen mit 1/200 bzw. 1/400 mol. Lösungen Hydroxylaminchlorid behandelt. Zwecks gleichmässigerer Dosierung hatten wir das Besprühen bereits durch das Tauchen ersetzt. Jetzt versuchen wir, eine exakte Dosierung durch Aufnahme der Lösungen über den Stumpf der Mittelrippe abgeschnittener Fiedern zu erreichen. Ausserdem werden wir neben Hydroxylamin auch noch Trifluoracetat und Thiosemikarbazid  $\text{NH}_2 \cdot \text{CS} \cdot \text{NH} \cdot \text{NH}_2$  Schmp. ca 181—182° C anwenden.

In den beiden ersten Versuchsreihen zeigten einige Pflanzen vorübergehend eine mosaikartige Fleckung und eine eigentümliche Kräuselung der Blätter. Das äussere Bild erinnerte an die Symptome der A-Virose. Die im Gewächshaus verbliebenen Kontrollpflanzen waren symptomfrei. Bedeutsam wurde das Phänomen aber erst dadurch, dass im Palisadenparenchym dieser Blätter fast anschliesslich ungewöhnlich lang ausgezogene Spindelkerne gefunden wurden, eine Kernform, die bei *Aquila* bis dahin noch nie angetroffen worden war — für diese Sorte sind in „gesunden“ Blättern kleine runde Kerne charakteristisch — und die wir nach allen bisherigen Erfahrungen als cytologisches Merkmal viröser Prozesse ansehen. Leider war es seinerzeit nicht möglich, die Versuche mit dem Nachbau dieser Pflanzen weiterzuführen. Nach mehr als 1½ jähriger Unterbrechung können die Arbeiten im Winter 1959 in vervollkommneter Form wieder aufgenommen werden.

## Diskussion

Es mögen nun noch einige Bemerkungen zur theoretischen Seite unseres Problems folgen. Wir können uns der Auffassung von der organismischen Natur der Viren nicht anschliessen, weil sie uns nicht mit dem vereinbar erscheint, was sich an Konsequenzen aus der Definition des „Lebendigseins“ ergibt. Auch der Versuch einer Einstufung zwischen „lebend“ und „unbelebt“ kann nicht befriedigen.

Unter Viren verstehen wir Eiweissverbindungen mit bestimmten Funktionen, die wesentlich nur in Wechselwirkung mit lebendem Substrat zustandekommen und im Endergebnis zur Synthese neuer Virussubstanz führen. Das Hauptcharakteristikum der Wechselwirkung ist eine Fehl- und Fremdsynthese im pflanzlichen Eiweisstoffwechsel, deren Richtung zwar durch die Viruskomponente angegeben, deren Ablauf und Endprodukt jedoch von der Pflanze gestaltet wird. Dementsprechend ist auch zu erwarten, dass das Syntheseprodukt quantitativ und qualitativ in hohem Masse von der Pflanze und zwar von ihrem Verhalten gegenüber den von der virösen Substanz ausgehenden „Informationen“ abhängt und mit dem provozierenden Virus nicht unbedingt identisch sein muss. Es ist immer damit zu rechnen, dass eine solche Identität durch die einstweilen zur Verfügung stehenden Methoden nur vorgetäuscht wird. Die Zweifel an der Identität von provozierender und produzierter Virussubstanz sind umso mehr berechtigt, und man könnte sagen notwendiger, je höher die Molekulargewichte liegen.

Die vorhin gegebene Definition für „Virus“ lässt auch die Möglichkeit zu, dass derartige Proteide von der Pflanze allmählich aufgebaut werden. Um ein Missverstehen auszuschliessen, sei noch einmal betont, dass wir jeden Gedanken an eine generatio spontanea von Viruspartikeln dabei ausschalten. Wenn wir Krankheiten als reversible oder irreversible Lebensprozesse im Grenzbereich der physiologischen Selbstregulierung betrachten, so gehört nach unseren Vorstellungen zu den irreversiblen Prozessen auch die endogene Virusbildung.

Eine solche Endogenese würde mit einer zufällig eintretenden, aber ätiologisch definierbaren Störung beginnen. Die späteren Auswirkungen dieser Störungen manifestieren sich in der Bildung von Substanzen mit immer grösseren Energiepotentialen und immer stärker ausgeprägten informativen Fähigkeiten und Funktionen bis zu solchen, die den eigentlichen Stoff- und Energiewechsel der Zelle im Sinne der Erhaltung des umsteuernden, zellfremd gewordenen Prinzips „um-informieren“.

So gesehen wäre die endogene Virusbildung die Folge einer Investierung von Energie an falscher Stelle.

Ohne Frage ist es einigermaßen schwierig, sich den Verlauf der Virogenese quasi aus dem Nullpunkt heraus im einzelnen vorzustellen, wenn die Fähigkeit zur Autokatalyse, zur Selbstreproduktion ausschliesslich den Nukleinsäuren und den Nukleoproteiden vorbehalten bleiben und das Produkt mit dem Autokatalysator wirklich identisch sein soll. Ein anderes Bild ergibt sich, wenn man nach GURWITSCH (1959) mit dem allgemeinen Vorkommen dieser Fähigkeit rechnen darf. Nach GURWITSCH treten bei Zusatz geringer

Mengen verschiedener Fermente zu den Lösungen einfacher Aminosäuren wie z. B. Glykokoll (für Alanin, Leucin, Histidin, Glutaminsäure, Tryptophan soll das gleiche gelten) Stoffe auf, die sich autokatalytisch bei wiederholten „Übertragungen“ unbegrenzt vermehren und die in gewisser Hinsicht den betr. Fermenten gleichen (l. c. S. 60 ff.)\*). SCHERSCHNEW (zit. n. Gurw.) hat sie „Fermentoide“ genannt. Bekanntlich hat EULER vor langen Jahren schon einmal von „Enzymoiden“ gesprochen.

Nach STERN (zit. bei Gurw.) soll ein und derselbe Eiweisstoff dank seinen verschiedenen, in seiner Struktur enthaltenen spezifischen Gruppierungen fähig sein, nicht nur eine, sondern mehrere katalytische Funktionen zu erfüllen.

Letzten Endes könnte wohl jede induzierte Veränderung der Energieverteilung zum Ausgangspunkt derartiger Reaktionsreihen werden. Es ist vorstellbar, dass Initialereignisse dieser Art in einzelnen Zellen eintreten. Da es eine funktionelle Gleichheit benachbarter Zellen nicht gibt (und bei der Vielzahl der beteiligten Komponenten auch gar nicht geben kann), so wird es von dem Verhalten der angrenzenden Zellen abhängen, ob die weiteren Reaktionen auf die Ausgangszelle beschränkt bleiben oder ob, bzw. mit welchem Ergebnis sie sich ausbreiten. Je näher beieinander sich solche Initialereignisse—zu denen im weiteren Sinne auch die Importation von Vollviruspartikeln gehört—abspielen und je schneller sie sich ausbreiten können, desto mehr Wahrscheinlichkeit besteht dafür, dass sie das Endstadium, die Bildung fertiger Viruspartikeln, erreichen.

Nach alledem ist die Möglichkeit einer Beschränkung viröser Prozesse auf eine einzige Zelle oder auch Zellgruppen einleuchtend. Folgerichtig wäre also prinzipiell mit dem Vorkommen viogener Mikroprozesse aller Entwicklungsstufen zu rechnen und zwar auch in Pflanzen, die als „gesund“ getestet worden sind. Die vorhin erwähnten zwei Keime von gesunden Knollen bestätigen das. Derartige Phänomene lassen sich weder durch Übertragung, noch serologisch oder mit Hilfe einer anderen der bisher gebräuchlichen Standardmethoden, sondern allenfalls mikroskopisch nachweisen.

Die hier vorgetragenen theoretischen Überlegungen und die daraus abgeleiteten Schlüsse lassen sich in folgender Arbeitshypothese zusammenfassen:

Die Virogenese wird im wesentlichen als ein physiologischer Prozess aufgefasst, den die Pflanze gestaltet.

Derartige Prozesse entstehen unter dem Einfluss akzessorischer Vollviruspartikeln. Sie könnten sich aber auch autonom aus Störungen des Eiweisstoffwechsels entwickeln (endogene Virusbildung) als „Fehlkataylsen“ im Sinne von MITTASCH.

Der Verlauf dieser endogenen Prozesse ist durch die (l. c. 135) fortschreitende Bildung von Eiweisskörpern charakterisiert, die das Stoffwechselgeschehen der betroffenen Zellen in zunehmendem Masse heteronom zu steuern vermögen und schliesslich im Sinne der eigenen Reduplikation umschalten.

Unter natürlichen Bedingungen dürfte die autonome Virogenese nur rel. selten zur Perfektion gelangen, da bei der allgemeinen Verbreitung der Voll-(Eu-) Viren eine vorzeitige Infektion eher zu erwarten ist.

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\*) Vgl. hierzu MITTASCH (1937).

Für den Verlauf der infektiösen Virogenese kann das etwa schon vorhandene, autonom gebildete Vorstufenmaterial in quantitativer und qualitativer Beziehung bestimmend werden.

Bei dem erwiesenen Vorkommen von virogenen und virösen Mikroherden ist es nicht möglich nachzuweisen, dass eine Pflanze völlig frei von virogenen oder virösen Prozessen ist. Vielmehr ist mit der Ubiquität solcher Prozesse zu rechnen.

Es genügt heute nicht mehr, Fakten zu beschreiben und zu registrieren, sondern darüber hinaus müssen Zusammenhänge gesucht und verstanden werden. Zu diesem Zweck ist es auch nötig, sich von bestimmten, methodisch gebundenen Anschauungen zu lösen, eine Neuorientierung zu versuchen und zur Diskussion anzuregen.

Eine Nachprüfung der Kernformenstatistik als diagnostisches Hilfsmittels zur Beurteilung der Virophilie ist erwünscht, weil uns diese Methode bei der Züchtung abbauresistenter Kartoffelsorten u. U. von Nutzen sein kann, vielleicht für eine Frühdiagnose viröser Krankheitsprozesse, die uns Aufschlüsse über die Virogenese selbst geben könnte, die aber z. B. bei der virologischen Kontrolle vegetativ vermehrter Kulturpflanzenbestände auch einen praktischen Wert hätte.

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- Die mit\* bezeichneten Arbeiten lagen nur im Referat vor.

*Anschrift des Verfassers:* Prof. Dr. Gerhard Staar, Phytopathologisches Institut der Friedrich Schiller-Universität, Jena, DDR

## К проблеме эндогенного образования вирусов

Герхард Штаар

Фитопатологический институт университета им. Ф. Шиллера, Ена, ГДР

## Резюме

В данной статье автор дает обзор работ, касающихся проблемы эндогенного образования вирусов. Цитологически измененные предшественники вирусных заболеваний симптомов вирусных заболеваний. В качестве таких цитологических признаков были, кроме прочего, описаны характерные клеточные включения из молодых проростков картофельных клубней. С их помощью можно доказать, что вирусные процессы могут иметь место и в клубнях, которые в опытах казались здоровыми. Неодинаковая встречаемость перетонированных клеточных ядер в падающих кончиках клубней картофеля расценивается как признак разной наклонности к вирусным заболеваниям (вирусофилия). В опытах, где вирусные заболевания вызывались с помощью физико-химических методов, наблюдалось значительно больше таких форм ядер в обработанных растениях.

Вирусогенез по существу истолковывается как физиологический процесс, который управляется растением и который может развиваться или автономно, или же после инфекции.

В связи с возможностью появления вирусогенных процессов и микрочаток вирусных заболеваний, которые можно анатомически выявить, доказательство того, что растение свободно от вирусов, становится невозможным. Более того, необходимо считаться с возможностью, что этот процесс является общераспространенным явлением.

## Dry Weight Increase of Leaf Disks as a Measure of Photosynthesis

JIRÍ BARTOŠ, ŠTĚPÁN KUBÍN and IVAN ŠETLÍK

Botanical Garden, Slovak Academy of Sciences, Košice

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### Souhrn

V práci se popisuje metoda, která umožňuje stanovit průměrnou intenzitu fotosynthesy vzorků asimilačního pletiva z poměrně velkého počtu listů. Měřítkem intenzity fotosynthesy je přírůstek sušiny v terčících z listové čepely, které se osvětlují po několik hodin za konstantních podmínek. Spolu s pokusnými vzorky se v jejich těsné blízkosti odebírají vzorky kontrolní, které slouží pro stanovení váhy sušiny terčků na počátku pokusu. Při vhodně zvoleném způsobu odběru a počtu vzorků neliší se průměrná váha sušiny terčků pokusných a kontrolních na počátku pokusu o více než  $\pm 0.5\%$ . Průměrný přírůstek sušiny v terčících o  $10\%$  může tedy být stanoven s přesností  $\pm 5\%$ . Pro dosažení této přesnosti jsme odebírali 200 až 500 terčků o průměru 8 nebo 12 mm do jednoho pokusu. Jsou však i jiné způsoby odběru, které dovolují dosáhnout této přesnosti. Terčíky se exponují v asimilační komoře o rozměrech ozážené plochy  $50 \times 40$  cm, kterou cirkuluje dostatečnou rychlostí temperovaný vzduch s konstantním obsahem  $\text{CO}_2$ . Komoře je shora osvětlena soupravou žárovek tak, že intenzita ozáření terčků je  $1,85 \cdot 10^6$  erg/cm<sup>2</sup> sec. Terčíky jsou v komoře uloženy do otvorů v plotně agarového gelu a tak jsou během pokusu zásobeny vodou. — Zjištěné hodnoty váhových přírůstků v prvních hodinách expozice lze považovat za intenzitu fotosynthesy terčků asimilačního pletiva charakteristickou pro zkoumané listy za daných podmínek. To plyne z pokusů, při nichž byly exponovány paralelní vzorky, a také z toho, že sušina terčků exponovaných v komoře přirůstá lineárně během prvních 6 až 9 hodin expozice při dané intenzitě ozáření. Intenzitu fotosynthesy terčků ve standardních a pokud možno optimálních podmínkách navrhuje nazývat fotosynthetickou kapacitou asimilačního pletiva zkoumaných rostlin.

### Summary

Isolated leaf disks are illuminated under constant conditions in a specially arranged chamber and their increase in dry weight within a certain time is taken as a measure of photosynthetic intensity. The weight increment is

estimated by comparing the dry weight of the illuminated disks with that of control disks sampled in the close vicinity of the former and dried immediately after sampling. Disks are taken from the leaf blades following a pattern which allows the average original weight of the control and experimental disk sets to be taken as identical within  $\pm 0.5\%$ . During illumination in the assimilation chamber the disks are inserted into circular openings bored in agar plates; thus, they remain satisfactorily turgid throughout the experiment, since they take up water through the section area from the gel. Air from the chamber circulates through a heat exchanger and the concentration of  $\text{CO}_2$  is maintained constant in the atmosphere. The chamber is illuminated from above by a set of light bulbs producing an illumination intensity of  $1.85 \times 10^5 \text{ erg/cm}^2 \text{ sec}$ . It was shown that the dry weight of the disks in the chamber increases proportionally with time for several hours. During this time the effect of injury caused through sampling as well as the conditions to which the disks are exposed in the assimilation chamber, apparently do not interfere with intense photosynthesis. Parallel samples from the same leaves give the same values of dry weight increase; the rate of photosynthesis (dry weight increments) estimated by this method, is thus characteristic for the leaves investigated.

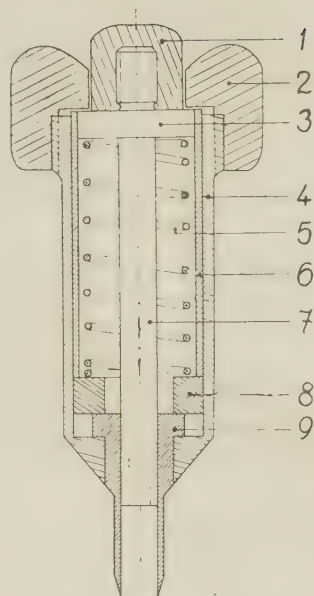
### Introduction

It was the aim of the work described in this paper to develop a method which would permit the estimation of the average intensity of photosynthesis in samples from a number of leaves under standard conditions. At the same time it was necessary for the method to be simple enough to render possible extensive comparative measurements to be carried out within a reasonably short time. Such a method opens the way to routine control in growing and breeding crop plants. The limits of accuracy of photosynthesis measurement should not exceed  $\pm 5\%$ . As standard conditions of exposure we selected those under which the maximum photosynthetic output of the photosynthetic tissue can be expected. It was considered particularly important to maintain full hydration of the leaf disks throughout the experiment and thus favour maximal aperture of the stomata, to bring the intensity of illumination to the level saturating photosynthesis, to maintain constant temperature within the range of  $25^\circ$  to  $30^\circ \text{C}$  and to supply sufficient carbon dioxide to the disks.

For measuring the intensity of photosynthesis the dry weight increase in disks from the leaf blade was used, the disks being sampled from the leaves and illuminated under constant conditions for several hours. In order to demonstrate that this principle can be applied to the measurement of photosynthesis it was essential to elucidate the following principal points:

- a) To prove that the estimation of dry weight increase can be carried out with the necessary accuracy and with a reasonable amount of work.
- b) To find an adequate method of supplying the isolated leaf disks with water during illumination so that they do not lose turgidity even under high intensities of irradiation.

Fig. 1. Schematic section through lever punch showing its construction. The handle (2) is connected with the mantle (4) by means of screw winding so that on screwing the flange the handle presses the inner mantle (6) onto a ring (8) which holds the knife (9) firmly in the opening at the lower end of the mantle (4). The piston (7) moves at its lower end through the knife cavity and at its upper end with its extended part (3) through the cavity of the inner mantle (6). The extended part (3) supports a spring (5) which holds the piston in its upper position. The piston is pressed with the thumb which rests on a push-button (1). The piston and the knife are exchangeable so that knife of lumen 8 and 12 mm. can be used. The punch is used for cutting out samples from leaves supported by a soft board stand while the knife cavity can take as much as 20—30 discs which are then extruded by the piston.



c) To demonstrate that the photosynthesis of isolated leaf disks in the chamber proceeds at a more or less steady rate and does not undergo any undefinable changes at least during illumination intervals producing a sufficiently large dry weight increment for precise determination. Irregularities might be expected due, for example, to injury at the cut edge of the disks or to specific conditions prevailing in the exposure chamber.

Moreover, it was necessary to verify whether the photosynthesis of samples under standard conditions is characteristic for the photosynthetic capacity of the leaves examined, i. e. whether the results obtained with disks sampled twice from the same leaves are satisfactorily reproducible.

It is proposed to call the intensity of photosynthesis in leaf disks under standard and optimal conditions the photosynthetic capacity of the leaves investigated. There are good reasons to assume that this value is not limited by factors other than the structure and biochemical characters of the plants under consideration.

## The Method

### 1. Sampling Technique

Disks are cut out from the leaf blade with the aid of circular punches in such a manner that stronger nervature is avoided. Punches of 8 mm. and 12 mm. bore are most suitable and three types of punch were constructed to this purpose: a simple borer with piston, a lever device and a tong-punch. Their construction is seen from Figs. 1, 2, 3 and 4, and the corresponding legend contains a full description of the individual parts.

The lever and tong-punches were constructed especially for sampling disks directly from leaves attached to living plants. In this case work with the borer-like punch is slower since the leaf must be supported by a soft sheet below the place of cutting. The lever punch is fastest and most reliable in handling and it is especially appreciated in work with tough leaves

with sturdy nervature, such as in maize; in such leaves it is rather troublesome to obtain a clear cut when the borer is used and the cutting edge of its knife must be very sharp. On the other hand it is rather difficult to select appropriately the right spot for cutting out the disks when using the lever punch. This is of great importance, however, if conjugate disks are taken (and visible nervature should not be interfered with). Since in this work disks were sampled most often on detached leaves, the greatest part of sampling (except maize) was carried out with the borer-punch. In this case the leaf is placed on a sheet of soft material which does not crumb (as is the case with cork or rubber). Sheets of softened polyvinylchloride were found to be most suitable to this purpose (see Fig. 5.).

Sampled disks are placed in test-tubes on stands (Fig. 6) and are dried therein for at least 6 hours at 105° C.

To weigh a large number of samples by the usual method in closed vials would be prohibitively time-consuming. It was shown by detailed examination that the amount of water absorbed by the dry matter of leaf disks during weighing does not exceed 0.1 to 0.2% of their dry weight and that it thus remains within the limits of permissible error. This finding holds for weighing on a semiautomatic analytical balance, where the entire operation does not take more than about 30 sec. On using a torsion balance the time of weighing can be reduced still further. The sample is removed from the desiccator, emptied from the test-tube into a vial and weighed immediately. For the simpler removing of a great number of test-tubes from the desiccator this was arranged as shown in Fig. 7.

## 2. Sampling Pattern

The methods of indirect estimation of dry weight which do not require drying of the object are not sufficiently accurate at the present time to reach the accuracy required in the present work. It was therefore necessary to use the conventional method of parallel samples for dry weight increment determination. The fundamental condition for the successful application of this method is to find two sets of disks conjugate one with another in such way that their dry weight would not differ by more than a required value, if compared immediately following sampling. It was stipulated in our work that the dry weight increment should be determined with an accuracy of at least  $\pm 5\%$ . We assumed further that it would be possible to attain a dry weight increment amounting to 10% original weight of the disks within a reasonable time. This implies that the dry weight of the experimental and control set of disks must not exhibit a significant difference of more than 0.5%.

In order to achieve this, we used the basic principle of SACHS' method which makes use of the fact that conjugate samples taken from the same leaf (or leaves) are mutually much more identical in their dry weight than samples taken at random. To this aim, parallel samples (for the experimental and control sets) are usually punched out symmetrically with respect to the main nerve from each half of the leaf. In this case, however, the weight asymmetry in the two leaf halves comes into effect, increasing the variability in dry weight of conjugate samples. We have found that it is much more suitable to sample disks for the conjugate sets in close vicinity to one another, on different parts of the whole leaf.

In addition to these fundamental principles, the sampling pattern used most often during this work has been determined by two more formal circumstances:

a) At the beginning of work an analytical balance was used with reliable reading of at most 0.1 mg. Individual dried disks could not, therefore, be weighed with sufficient accuracy, the dry weight of one disk 8 mm. in diameter amounting 1 to 2 mg. according to the character of the leaf. We decided therefore to weigh groups containing *cca* 20 disks together; thus every possibility was excluded of inaccurate reading during weighing, which would produce an error exceeding 0.5%.

b) Since the method was required to render a possible comparison of photosynthetic capacity of individual varieties and species of crop plants, the size of the sample taken into one experiment and the sampling pattern should be modified so as to give an average and representative sample of the photosynthetic tissue in the population examined. Several leaves from a greater number of plants should enter the representative sample and the resulting great number of disks required some principle of grouping, if the weighing was not to be excessively time-consuming. We decided to group disks from a single plant and weigh them as one weighed sample; this contained 18 to 24 disks.

The following sampling pattern was often used when comparing whole populations: three leaves were selected on a plant and a total of 18 disks were sampled from them (three disks from each leaf half) for the experimental sample and another 18 for the control weighed sample. Twenty-four such samples (18 disks each) could be placed in the apparatus so that a total of 18 times 24 disks constituted the experimental and control sets; 24 plants could be examined in a single experiment.

It should be emphasized that this number of samples is quite arbitrary and that it is possible to work with completely different arrangement of groups of disks as will be shown in another paper.

### 3. The Apparatus

During the exposure of disks for a long enough time at a relatively intense illumination the main difficulty is to keep the disks in a turgescient state. Only after this problem had been solved successfully, could an apparatus of suitable construction be devised.

The disks absorbing radiation transpire rapidly and they lose their water content within a short period of time. This holds even for disks floating on the water surface if their cut edge is not wetted. Moreover, the placing of disks on the water surface or on a wet material during photosynthesis measurement cannot be recommended because free gas exchange through stomata on the lower surface of the disks is thus interfered with.

A suitable solution of this problem is the one in which the disks are supplied with water from the cut edge, i.e. they are placed into a matrix of porous material soaked with water. The matrix is provided with openings corresponding in size to the diameter of the disks. Of various available materials tested, filter paper and various tissues proved unsuitable as the fine fibres from the tissue edges adhere to the disks when the latter are removed from the matrix after the experiment. A suitable method was finally found in the application of 5% agar gel from which

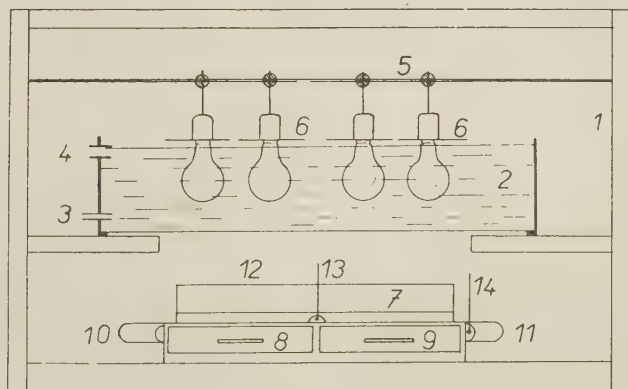


Fig. 8. Schematic section through the exposure chamber and the illumination system of the apparatus. The wooden frame (1) on the bottom of which the exposure chamber is placed carries a sheet-metal jar (2) with glass bottom into which water passes from below (3) and leaves through an overflow (4). Into the water are submerged bulbs fixed in sockets attached to a steel construction (5). The 500 W Teslafof B bulbs are provided with reflection plates (6) and are distributed so as to produce the maximum uniformity of illumination in the chamber. Above the chamber there are 3 rows of 4 light bulbs each, one of them being shown in the Figure. The uniformity of illumination at the chamber edges is improved by a short light channel made of bands of mirror glass (7). The exposure chamber has a double bottom in the space of which temperating water circulates, and two spaces (8, 9) into which shelves with disks can be inserted (11, 12). Into these spaces air is brought from distributors at the chamber walls (10, 11) and removed through the duct (13) in the chamber centre.

thin plates were poured out (3—5 mm. in thickness). In these plates openings were made with a punch, the diameter of which was 0.2 mm. smaller than that of the punch for disks. The plates were placed on a support of thin wire netting so that both sides of the disks were in adequate contact with the atmosphere. The disks were placed into the slightly conical openings in the agar so that good contact between the cut edge and the agar was ensured. If the agar was sufficiently wetted it was able to supply the disks with water even at high intensity of illumination so that even after several hours of exposure the disks were completely turgescient.

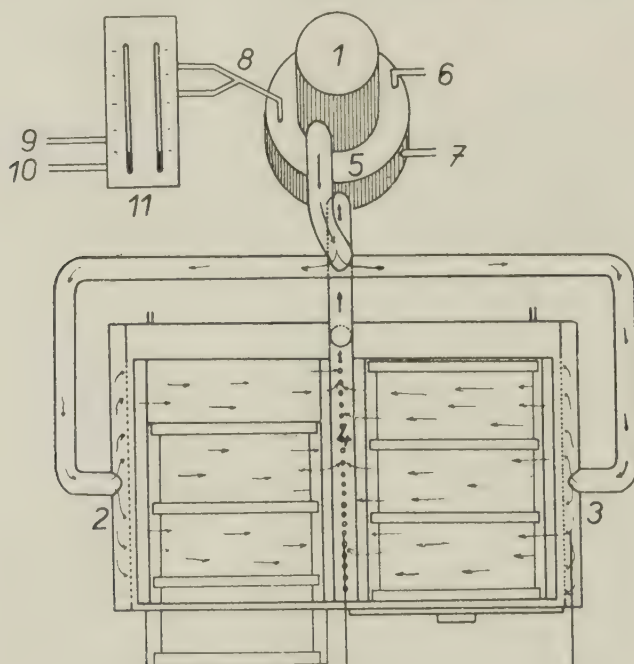


Fig. 9. Schematic drawing showing the circulation of air through the exposure chamber. The air is pumped by a ventilator (1) into spaces at chamber walls (2 and 3) from which it is distributed through two rows of holes over the entire width of the chamber. In the centre of the lid there is a row of holes leading into the space (4) from which air is sucked over a condenser (5) back to the ventilator. The condenser is tempered by running water from the thermostat (6, 7 inflow and outflow of water). In the condenser space an auxiliary stream of air (8) from a compressor enriched with  $\text{CO}_2$  is introduced. The amount of air and  $\text{CO}_2$  added (9 and 10) is measured by flow meters (11).

A disadvantage of this method consists in the fact that agar plates lose a part of their original water content on repeated use and cannot be quite efficiently regenerated. It is therefore necessary, approximately after every 12 hours of exposure of agar to prepare new plates. A second drawback of the described technique lies in the fact that only a sufficiently thick agar plate will fulfil its task satisfactorily. The disks must be pushed into the openings relatively deep so that they are firmly enclosed by the agar gel. This brings about a partial shading of the disk edges since light does not reach the plates only perpendicularly, due to the technical arrangement of the illumination system. This will somewhat alter the light intensity.\*)

\*) The publication deals with experiments carried out in the years 1957 and 1958 when agar plates were used. Later, it was found that matrices devoid of the above shortcomings can be prepared from spongy polyurethane.

The construction of the apparatus was modified several times. The resulting solution which satisfactorily met most requirements placed on it is shown in Fig. 8. and Fig. 9. The apparatus consists of three main parts; the exposure chamber, the illumination system and the arrangement for air-conditioning of the chamber.

The **exposure chamber** is a flat box made of polyvinylchloride sheets and covered with a plexiglas lid. The chamber has a double bottom in which tempered water circulates. From the side walls an air stream is passed into the chamber, the stream being uniformly distributed

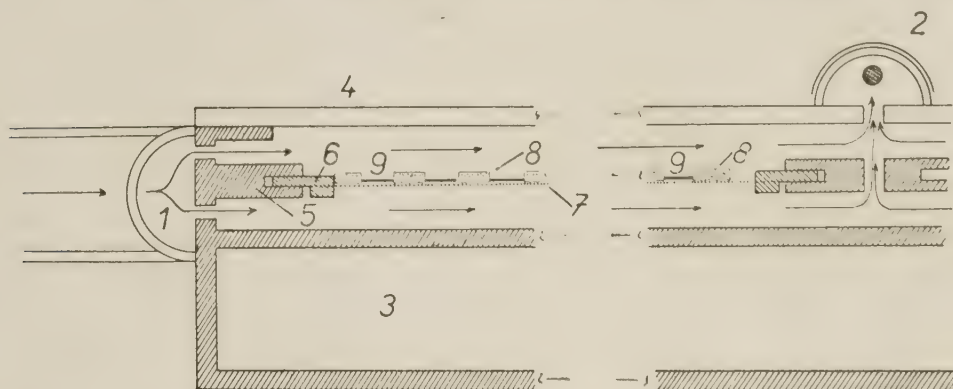


Fig. 10. Schematic cut through a half of the exposure chamber showing in detail the movement of air through the chamber and the position of shelves. 1 — tube distributing air along the chamber walls; 2 — tube removing air from the chamber; 3 — space for tempering water; 4 — plexiglas chamber lid; 5 — grooves for shelves; 6 — side ledges of shelves; 7 — bottom of shelf from wire netting; 8 — agar plate; 9 — disks. Circulation of air is shown by arrows.

over the entire width of the box by means of holes bored out in the side walls (see Fig. 10). The air is removed from the chamber through a number of holes made in the centre of the lid. Thus the chamber is functionally divided into two halves and into each one of them a shelf can be fitted containing the agar plates with experimental disks (Fig. 11, 12). Each shelf is divided into three sections, in which agar plates with the matrix of openings are placed. In the experiments described here each plate usually contained  $6 \times 12$  or  $8 \times 16$  disks. The plates are separated by grooves filled with water. Water is led from the groove to the edges of the agar plate by means of a filter paper strip which is submerged at one end into the groove while its other end lies close to the plate edge along its entire length (Fig. 11). Above one end of each groove the chamber lid is provided with tubes through which the grooves are refilled with water without the necessity for opening the chamber.

The bottom of the shelves is made of thin brass wire netting and covered with a film of the epoxide resin PGA 40. The shelves are placed in the chamber with their bottom in the middle of the chamber height. The air stream thus flows uniformly below and above the disks and supplies  $\text{CO}_2$  to stomata on both surfaces. One of the shelves is provided with 12 pairs of thermocouples. Their warm connections were hammered out into a thin foil about  $1 \times 3 \text{ mm}^2$  in size and were placed directly above the wire bottom of the shelf, at places where the agar plate contained openings for the disks. The disks placed into the openings were therefore in good contact with the warm connections (Fig. 12).

**The illumination system.** A sufficiently intense and simultaneously uniform, illumination of the relatively large area ( $450 \times 550 \text{ mm}^2$ ) where the disks are placed is not easy to achieve. If it is to be obtained by ordinary means it is simplest to illuminate the area with a large number of light bulbs with super-heated filament. Twelve 500 W Teslaphot B light bulbs with dull glass are placed over the exposure chamber and submerged in water up to the socket, the

whole set being placed in a jar with a glass bottom. The jar is filled with circulating tap water so that adequate cooling of the bulbs is ensured and their life is prolonged. Between the jar bottom and the light bulbs remains a layer of some 5 cm. of water which absorbs infrared radiation. On the sockets of the light bulbs white-painted metal sheets are mounted which touch each other and form a uniform reflection area.

Uniformity of illumination over the entire area is achieved mainly by the correct spacing of the light bulbs and further improved by using a short light channel which levels out illumination especially at the edges of the illuminated area. When the light bulbs are supplied with 210 V (nominal voltage is 220 V) it is possible to attain a light intensity of  $1.85 \times 10^5$  erg./cm<sup>2</sup>. sec. in the plane where the disks are placed. Differences between the measured values of light intensity never exceeded 5%. The illumination system was connected to mains through 2000 W stabilizers eliminating voltage fluctuation.

**Air-conditioning of the chamber.** The chamber air was pumped by a centrifugal ventilator through a heat exchanger with controlled temperature and was then passed back into the chamber. The output of the ventilator decreased by the resistance of the connecting tubes and of the chamber is estimated at 200 l./min. which corresponds to a linear velocity of air in the chamber of 10 cm./sec. Into this closed circulating system an auxiliary stream of air is fed continually, thus ensuring sufficient and uniform concentration of carbon dioxide. This auxiliary stream of air moved at a velocity of 500 l./hour and carbon dioxide was added to it at different volume ratios. Air-conditioning was not perfect in the apparatus described; there was a difference of 3–4° C between the temperature of incoming and outgoing air which was due to the high intensity of radiation entering the chamber. The temperature gradient was kept constant so that it did not affect the experimental results unpredictably. With the aid of suitable distribution of the disks it was possible to decrease the effect of the gradient to a minimum when comparisons were made within a single exposure.

#### 4. Material and procedure

The populations of plant varieties used in this work were grown under comparative conditions on experimental plots in a soil of medium bonity and in the moderately aride climate of Košice, Eastern Slovakia.

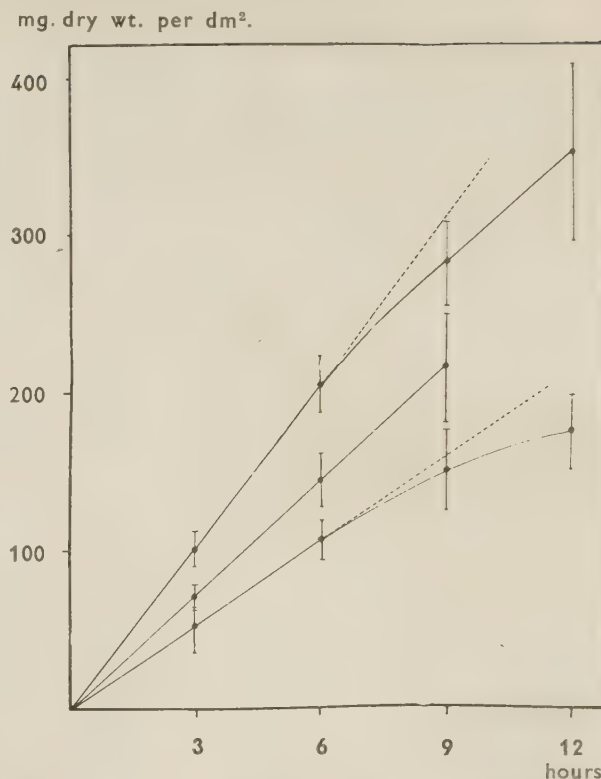
The leaves designed for sampling were detached from the plants early in the morning and, with their petioles dipped in water, they were stored in a dark room for 12 to 24 hours to reach good turgidity. Afterwards disks were punched out from the leaves with the borer (see Fig. 5), the control sets were placed immediately into the test-tubes for drying (see Fig. 6) and the experimental ones were distributed into the openings of the matrix agar plate (see Fig. 11). The whole procedure of sampling and preparation of the disks for the experiment lasted 45 to 75 minutes, when two persons cooperated.

#### Results

In order to show that the values obtained by the described procedure can serve as a reliable measure of photosynthetic capacity of the leaf tissue investigated it was necessary to demonstrate that the amount of accumulated dry matter is not affected by accidental factors. As mentioned in the introduction, it was necessary to verify that the disks exposed will accumulate the assimilates at a sufficiently constant rate during the time used for exposure and that the values obtained with the same material are reproducible.

The time course of dry weight accumulation in isolated leaf disks of various crop plants was examined in a number of experiments. Fig. 13 gives a graphical summary of the results of three such experiments which may serve as an example; details of the performance of these experiments were as follows: —

Fig. 13. Curves showing the increment in dry weight of exposed disks with time. Upper curve: results of experiment 10-1-58 with fodder cabbage (Coulet de Flandre variety). Central curve: results of experiment 4-3-58 with sunflower (Slovenská sivá variety). Lower curve: Results of experiment 4-1-58 with maize (Český bílý koňský zub variety). Vertical intercepts show the range of dispersion  $\pm t_{0.025} \cdot s_{\bar{x}}$  representing the limit of significance for 5% probability.



**Experiment 10-1-58.** Date: August 20, 1958. Material: fodder cabbage (*Brassica oleracea* var. *acephala* variety Coulet de Flandre.) The leaves used for the experiments were cut off in the field at 6:30 a.m. and placed with their petioles submerged in water for 4 hours in a dark chamber. After that period the samples were removed. A total of 24 plants were used for the experiment, 3 large healthy leaves being removed from each. From each leaf, 6 experimental and 6 control discs were cut out, using a punch 8 mm. in diameter. Three times six experimental disks originating from a single plant were placed in the apparatus in a row and formed one weighed sample. There were 24 such rows in the apparatus, i. e. 12 on each shelf. After 3 hours of exposure the disks placed in rows 1, 5, 9, 13, 17, 21 were removed; after 6 hours of exposure those from rows 2, 6, 10, 14, 18, 22, and analogously disks were removed after 9 and 12 hours of exposure. The value of dry weight increment after 3, 6, 9 and 12 hours thus represented an average of the weight difference of six corresponding—experimental and control—groups of disks. — Temperature of the air in the exposure chamber: entry 21° C, exit 26° C. According to the readings of the thermocouples the temperature of the disks was on an average 2° C higher than that of the air in the chamber. The average temperature of the disks in the chamber was thus 25.5° C.

**Experiment 4-3-58.** Date: August 8, 1958. Material: Maize (*Zea mays* L., variety Koňský zub bílý). The leaves used for the experiment (3 leaves from each of 24 plants) were cut off at 7:0 a. m. in the field and placed in a dark room with their bases submerged in water for 10 hours. The programme of sample removal from the leaves, their placing in the chamber and the time schedule of removing the individual samples were the same as in the experiment 10-1-58.

**Experiment 4-1-58.** Date: August 2, 1958. Material: sunflower (*Helianthus annuus* L., variety Slovenská sivá). Leaves used for the experiment were cut off at about 7 a. m. in the field and placed for 24 hours in a dark room with their petioles submerged in water (3 leaves from each of 24 plants). The further procedure was the same as in the two preceding experiments. A punch of 12 mm. in diameter was used. Atmosphere: air + 1% CO<sub>2</sub>. Temperature of air in the chamber: entry 26° C, exit 31° C, average temperature of disks in the chamber 30.5° C.

In order to illustrate the variance characteristic of the primary data obtained with this method, Table 1 shows the results of another experiment *in extenso* including their statistical treatment.

Table 1. Dry weight increments of leaf disks of fodder cabbage in dependence on time. Result of experiment 20-1-58

 $W_1$  — dry weight of control samples $W_2$  — dry weight of experimental samples dried after exposure $d = W_2 - W_1$  — dry weight increment during exposure

| Exposure 3 hours |       |     | Exposure 6 hours |       |      |
|------------------|-------|-----|------------------|-------|------|
| $W_1$            | $W_2$ | d   | $W_1$            | $W_2$ | d    |
| 43.2             | 52.7  | 9.5 | 42.5             | 61.6  | 19.1 |
| 46.8             | 56.6  | 9.8 | 47.6             | 69.2  | 21.6 |
| 45.4             | 54.4  | 9.0 | 45.2             | 64.0  | 18.8 |
| 49.0             | 58.0  | 9.0 | 47.5             | 65.6  | 18.1 |
| 52.3             | 61.5  | 9.2 | 52.4             | 72.4  | 20.0 |

| Exposure 9 hours |       |      | Exposure 12 hours |       |      |
|------------------|-------|------|-------------------|-------|------|
| $W_1$            | $W_2$ | d    | $W_1$             | $W_2$ | d    |
| 42.0             | 69.6  | 27.6 | 43.4              | 78.2  | 34.8 |
| 46.4             | 74.5  | 28.1 | 46.5              | 80.1  | 33.6 |
| 44.6             | 71.4  | 26.8 | 43.8              | 72.0  | 28.2 |
| 47.0             | 72.1  | 25.1 | 47.5              | 75.8  | 28.3 |
| 42.6             | 65.4  | 22.8 | 43.5              | 69.7  | 26.2 |
| 55.7             | 80.8  | 25.1 | 54.4              | 83.3  | 28.9 |

| Exposure hours                            | 3              | 6               | 9               | 12              |
|-------------------------------------------|----------------|-----------------|-----------------|-----------------|
| $\bar{d} \pm t_{0.025} \cdot S_d$         | $9.3 \pm 0.31$ | $19.3 \pm 1.36$ | $25.9 \pm 2.05$ | $30.0 \pm 3.54$ |
| V                                         | 0.09           | 1.66            | 3.88            | 11.56           |
| n                                         | 6              | 6               | 6               | 6               |
| Smallest significant difference (in %)    |                |                 |                 |                 |
| $P < 0.05$                                | $\pm 3.4$      | $\pm 7.0$       | $\pm 7.9$       | $\pm 11.8$      |
| Dry weight increment in % original weight | 19.8           | 41.3            | 55.8            | 64.5            |

In the **experiment 20-1-58** the time dependence of dry weight increment in disks from leaves of fodder cabbage was examined, as in the experiment 10-1-58, the results of which are plotted in Fig. 10. The conditions of this experiment were also completely identical with those of 10-1-58, with the exception that the experiment 20-1-58 was carried out a month later, on September 27, 1958.

It follows from the results shown above that the dry weight of disks increases uniformly up to 6 hours of exposure at the given light intensity. In the experiments shown in Fig. 13, this corresponds to the following increments of dry weight (referred to average dry weight of samples before experiment and expressed in round numbers): fodder cabbage 30%, sunflower 45%, maize 25%. After 9 hours the results are still quite proportional in sunflower,

Table 2. Comparison of dry weight increments of two samples removed in parallelly

| Sample No.                                                      | Group A | Group B | Sample No.      | Group A         | Group B |
|-----------------------------------------------------------------|---------|---------|-----------------|-----------------|---------|
| 1                                                               | 20.9    | 19.4    | 7               | 20.3            | 19.8    |
| 2                                                               | 22.3    | 22.6    | 8               | 18.8            | 18.6    |
| 3                                                               | 19.5    | 19.2    | 9               | 17.8            | 17.2    |
| 4                                                               | 20.4    | 19.9    | 10              | 13.0            | 12.9    |
| 5                                                               | 19.5    | 18.1    | 11              | 17.6            | 18.1    |
| 6                                                               | 19.0    | 18.6    | 12              | 19.9            | 20.4    |
| Results of statistical treatment:<br>Group                      |         |         | A               | B               |         |
| $d \pm t_{0.025} \cdot s\bar{d}$ (mg./20 cm <sup>2</sup> 3 hr.) |         |         | $19.1 \pm 1.47$ | $18.7 \pm 1.45$ |         |
| V                                                               |         |         | 5.35            | 5.28            |         |
| n                                                               |         |         | 12              | 12              |         |
| Smallest significant differences in %<br>for $P < 0.05$         |         |         | 7.7             | 7.8             |         |
| t-Test for the difference between A and B                       |         |         | $t_d = 0.485$   | $P = 60\%$      |         |

there is a slight deviation in maize and in fodder cabbage the deviation from linearity is very marked.

The reproducibility of the results was verified in several differently arranged experiments. As an example, Table 2 contains the results of one such experiment and their statistical treatment *in extenso*.

The conditions of the experiment 3-6-58 were as follows. Date: August 1, 1958. Material: kohl-rabi (*Brassica oleracea* var. *gongyloides*, early variety). Experimental arrangement: 3 leaves from each of 12 plants were cut off in the field at about 7 a. m. They were then placed for 24 hours with petioles submerged in water in a dark room; after that the removal of samples was begun. From each leaf 6 control disks were cut out at first and all samples from one plant (i. e. a total of 18 disks) were taken in one weighing. A punch of 12 mm. in diameter was used. In the close vicinity of each control disk two samples were taken for exposure which are marked *a* and *b*. All *a* samples from one plant formed a single weighed experimental set A, all the *b* samples were combined in set B. There were thus  $2 \times 12$  of such sets; the sets A and B removed from the same plant were always placed in the apparatus in adjacent rows. The exposure of disks lasted for 3 hours. Atmosphere: air + 1% CO<sub>2</sub>. Temperature in the chamber: entry 20°C, exit 26°C, average temperature of samples 25°C.

It follows from the results that between the values of group A and those of group B no statistically significant difference can be demonstrated even for  $P = 0.05$ . The identity of the two groups is, in addition to the statistical parameters, established by some individual cases, such as are for example samples 2 and 10. This instance is mentioned here, although deviations from the mean as marked as they are in sample 10 are not typical of the great majority of the results obtained by the method described.

## Discussion

The methods of measurement of the photosynthesis rate based on dry weight accumulation are usually considered as rather inferior and somewhat outdated. A measurable increment in dry weight can be achieved only after a relatively long period of time and rapid changes in the course of photosynthesis cannot be investigated by this method. In addition, the lack of success of many experiments applying Sachs' method to the study of photosynthesis gave rise to the opinion that the dry weight increment methods are not sufficiently accurate. This negative attitude, however, is not justified.

Sachs' method in its original form (SACHS 1840) and in many variants (THODAY 1909, KOSTYTSHEV et al. 1926, DENNY 1930, PICKETT 1936, PORTER 1937, BORODULINA and KOLOBAYEVA 1953) possesses an inherent error which considerably limits its serious application or makes it altogether impossible. This consists in the translocation of assimilates out of the leaf blade during the experiment; well-based objections can be raised to all the suggested methods of correction. This limitation, however, has no bearing on the actual possibility of determining the dry weight changes per unit leaf area with sufficient accuracy. The estimation of dry weight increment is considered as inaccurate mainly on account of the fact that it was mostly carried out without proper statistical treatment of results. In the few cases where this was not so, very good results were obtained (PORTSMOUTH 1949). If all the possible—and frequently insufficiently respected—sources of error in gaseometric methods are taken into consideration then the accuracy of  $\pm 5\%$  obtained here in measuring the intensity of photosynthesis from the dry weight increment is certainly satisfactory. The translocation of assimilates cannot distort the results of photosynthesis estimation as long as the dry matter accumulation is measured directly in samples from the leaves, i.e. by the method described in the present paper.

It is the aim of the present paper to show first of all that the method of dry weight increase yields results comparable with other methods in accuracy, if samples are taken in a suitable manner. This fact is considered to be important as the amount of organic substances formed represents a more direct and probably more reliable indication of the capacity of the photosynthetic apparatus than, for example, the amount of  $\text{CO}_2$  absorbed (NICHOROVICH 1955). In proceeding one step farther it can be said that the increase in caloric value, stored in the form of chemical bonds, represents the most important criterion of photosynthetic yield and efficiency. The method described makes it possible to apply even such calorimetric evaluation. Comparison of the output of the photosynthetic tissue of leaves of different species and varieties of crop plants represents one of the most fundamental problems when the question of photosynthesis is considered from the practical point of view.

Leaf disks had been used previously mostly for the study of synthetic processes (especially the feeding experiments with floating leaf disks), of active uptake of substances and of water deficit (a review can be found in YARWOOD 1946, further see e.g. WEATHERLEY 1950, PENSELL and WEATHERLEY 1958). Photosynthesis in leaf disks has so far been measured only by manometric

methods (a review in ZALENSKY, SEMIKHATOVA and VOZNESENSKI 1955). As long as these methods serve the study of quantum yield of photosynthesis or involve use of a relatively weak light intensity with a corresponding low intensity of photosynthesis, manometric methods can be as good as any other method. In measuring the photosynthesis at higher radiation intensities various difficulties arise. When the leaf samples rest on a support in the gaseous phase, they necessarily lose water by evaporation; if they float on the water surface they are, as shown above, supplied with water in a rather undefinable manner and in addition, the pores on one side of the disk are blocked with water. The gravest objection to a great deal of work carried out on leaf disks by the manometric method, however, consists in the fact that the supposed equilibrium concentration of carbon dioxide has not been reached in the gaseous phase (see ZALENSKY, SEMIKHATOVA and VOZNESENSKI 1955). Since the liberation of  $\text{CO}_2$  from bicarbonate buffers is considerably slower than has been assumed heretofore, the area of leaf sample that can be placed in one flask is rather restricted. Comparative measurements on a larger scale thus become practically impossible.

The method and the apparatus described in this paper make it possible to determine the photosynthesis of disks of photosynthetic tissue without the limitations mentioned and thus to examine freely the influence of various factors on the stationary rate of photosynthesis.

In order to facilitate the work with the apparatus, however, and particularly in order to ensure routine work, it will be necessary to remove several shortcomings of a technical nature.

Most results presented in this paper were obtained with a smaller number of weighed samples (6 or 12 samples of 18 disks each) than was found necessary at the outset of the work, to achieve the sufficient accuracy of  $\pm 5\%$  (i.e. 24 samples). In spite of this it may be seen that even with a decreased number of samples the smallest significant difference remains within reasonable limits (mostly below 10%).

Some facts observed in the course of our work are of importance not only in connection with the method described but they have a farther reaching significance. The good reproducibility of results does not show only that the increase in dry weight of isolated leaf disks can be used for the characterization of photosynthesis in the tissue of leaves investigated but it also indicates that the photosynthetic tissue of healthy leaves is quite homogenous as far as its photosynthetic capacity is concerned. Surprisingly small samples of photosynthetic tissue (e.g. 6 disks sampled from each of 3 leaves on one plant) can apparently be taken as representative. Further, uniform increase in disk dry weight during the exposure up to high percentages of the original dry weight demonstrates an unexpectedly high ability of the photosynthetic tissue to accumulate assimilates. If the increments are linear for 6 hours under conditions favourable for intense photosynthesis it appears improbable that an accumulation of assimilates in the leaves after several hours of isolation could bring about a slowing down or blocking of photosynthesis under field conditions as has been assumed by several authors (KURBANOV 1933, WENT 1958).

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Addresses: Jiří Bartoš, Prom. Biol., Štěpán Kubín, Prom. Biol. and Dr Ivan Šetlík, Třeboň, Dukelská 145



Fig. 2. Dismantled and composed manual punch with exchangeable parts. Numbers refer to the same parts as in Fig. 1, with the following changes: 7a — piston of 8 mm. diameter; 7b — piston of 12 mm. diameter; 9a and 9b — knives of 8 and 12 mm. lumen, respectively; 10 — knife sharpener (here shown for the 8 mm. knife)

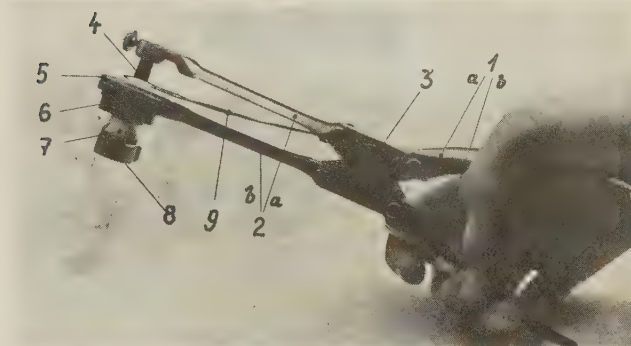


Fig. 3. Tong punch. The joint mechanism (3) which connects the tong jaws (2a, b) with the handles (1a, b) is constructed in such a way that on closing the tongs the jaws move in parallel. The knife (7) cuts out the disks against a matrix (5) which contains a hole corresponding to the knife diameter. Both the knife and the matrix are exchangeable. Into the block (6) on the lower jaw a hollow conical shaft (7) is introduced, the shaft carrying a holder (8) for a test-tube in which samples are collected. In order to prevent the leaf blade from adhering to the knife after cutting out the disk it is pressed back by a spring (9).

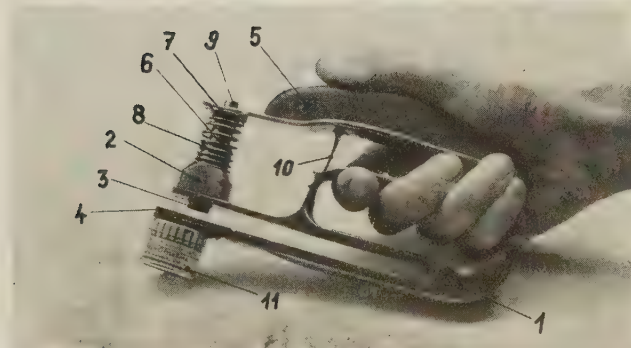


Fig. 4. Lever punch. The punch base (1) has the form of a U lying on its side. On its upper arm the groove (2) for the knife (3) can be seen, on its lower arm the matrix (4) is placed. Both the knife and the matrix are exchangeable. On compressing the lever (5) the knife is forced into the opening in the matrix. The lever returns to its original position, activated by a spring (6) which is supported on the extended part (7) of the knife groove (8). Pin (9) of the knife groove then moves in a fork notch at the end of the lever. The upper extreme position of the lever is controlled by an arrestor catch (11). The disks are collected in a box (11) fixed at the lower arm.

Fig. 5. Cutting of disks with a manual punch.

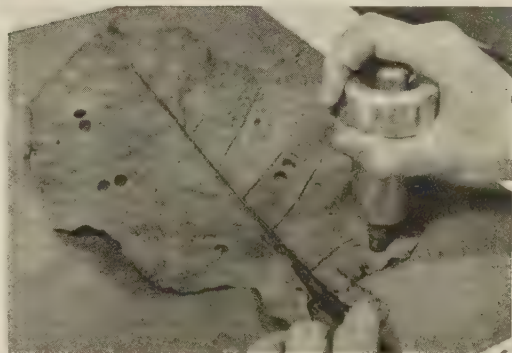




Fig. 6. Box with test-tubes in which the disks are placed for drying. The sequence of test-tubes determines the sequence of samples so that no special designation is required.

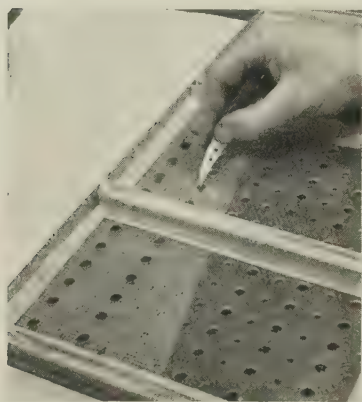


Fig. 11. Placing of disks into agar matrix. The wire netting bottom of the shelf and strips of filter paper supplying water from grooves to agar plates can be seen. In the experiment from which the figure is drawn, dry weight increments of disks of 8 and 5 mm. diameter were compared. In other experiments all the agar plate openings were of the same diameter.

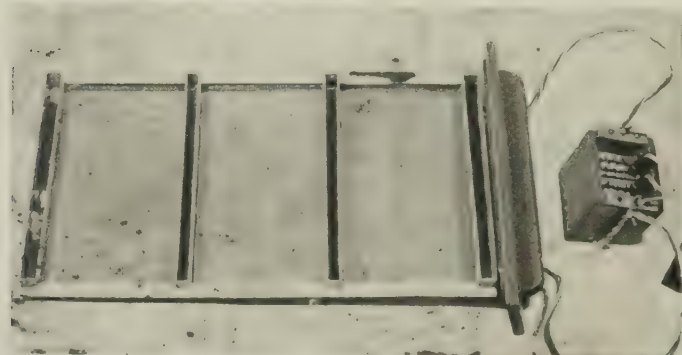
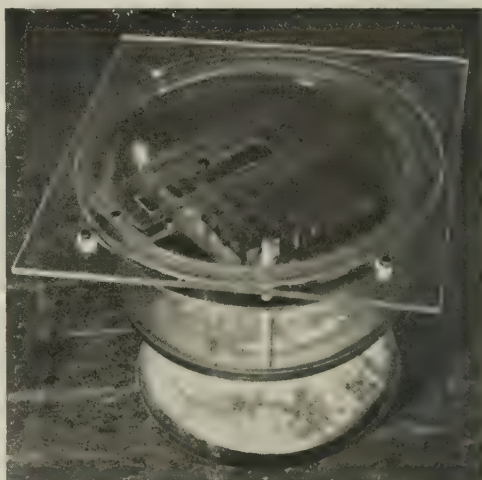


Fig. 12. Shelf provided with thermocouples for measuring relative temperature of disks. The thermocouple connections are placed under the side ledges up to the front of the drawer, the ledges being painted white so that they would not be heated much by the radiation.

Fig. 7. Modification of a desiccator facilitating operations during weighing dried samples. The desiccator lid is replaced by a thick plexiglas plate. The plate has a radial opening which is closed by a slide valve lubricated with vaseline. The box with test-tubes is placed in the desiccator on an elevated support so that the test-tube rims are about 1 to 2 cm. below the lid. By gradually turning the lid the radial opening can be brought to such position that any test-tube can be removed through it. The operation is so quick that it reduces the amount of atmospheric humidity penetrating into the desiccator to a minimum.



# Оценка интенсивности фотосинтеза по приросту сухого вещества в дисках ассимиляционной ткани

Иржи Бартош, Штепан Кубин и Иван Шетлик  
Ботанический сад Словацкой Академии Наук, Кошице

## Резюме

В работе описывается метод, при помощи которого возможно установить среднее значение для интенсивности фотосинтеза проб ассимиляционной ткани из сравнительно большого числа листьев. Мерой интенсивности фотосинтеза является прирост сухого вещества в дисках пластинки листа, освещаемых в течение нескольких часов в постоянных условиях. Вместе с опытными пробами отбираются из их непосредственного соседства и пробы контрольные, необходимые для установления сухого веса дисков в начале опыта. При правильно избранном способе отбора и числе проб средний вес сухого вещества дисков контрольных и опытных в начале опыта не отличается больше чем на  $\pm 0,5\%$ . Средний прирост сухого веса в дисках на  $10\%$ , следовательно, возможно установить с точностью  $\pm 5\%$ . Для того, чтобы достигнуть такой точности, мы отбирали от 200 до 500 дисков диаметра 8 или 12 мм в одном опыте. Существуют, однако, и другие способы отбирания, позволяющие достигнуть такой точности. Диски экспонируются в ассимиляционной камере с размерами освещаемой площади  $50 \times 40$  см, в которой с достаточной скоростью передвигается воздух определенной температуры и постоянного содержания  $\text{CO}_2$ . Камера сверху освещается набором лампочек так, чтобы интенсивность освещения дисков равнялась  $1,85 \cdot 10^5 \text{ erg/cm}^2 \text{ sec}$ . Диски в камере помещены в блок агарового геля и таким образом запасаются водой. Установленные значения прироста веса в первых часах экспозиции возможно считать интенсивностью фотосинтеза дисков ассимиляционной ткани характеризующей исследуемые листья в данных условиях. Это вытекает из опытов, в которых экспонированы параллельные пробы, и также из того, что сухое вещество экспонированных в камере дисков в течение первых 6—9 часов при данной интенсивности освещения растет линейно. Интенсивность фотосинтеза дисков в стандартных и, насколько возможно, оптимальных условиях, предлагаем называть фотосинтетическим капацитетом ассимиляционной ткани исследуемых растений.

## The Seed Progeny of *Solanaceae* Chimeras

VLADIMÍR FUČÍK

Department of Genetics, Faculty of Science, Charles University, Praha

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### Souhrn

Je popsáno získání dvou typů chimér mezi rajčetem a lilkem černým (*Solanum koelreuterianum* WINKLER a *S. gaertnerianum* WINKLER).

V mikromorfologických a anatomických znacích u *S. gaertnerianum* nebylo zjištěno žádné průkazné specifické ovlivnění jednoho pletiva druhým.

Semenné potomstvo chiméry *S. gaertnerianum* se ve všech studovaných znacích shodovalo s kontrolním lilkem.

Sledovány byly znaky: Velikost průduchů, tvar průduchů, tvar trichomů, pokožka plodů, průřez řapíkem, průřez listovou čepelí a počet semen.

### Summary

A description is given of the production of two types of chimera between tomato and nightshade (*Solanum koelreuterianum* WINKLER and *S. gaertnerianum* WINKLER).

In the case of *S. gaertnerianum* the micromorphological and anatomical characters showed no signs of the significant specific reciprocal effect of one tissue on the other.

The seed progeny of the *S. gaertnerianum* chimera resembled the control nightshade plants in all the characters investigated.

The following characters were studied: Size of stomata, shape of stomata, shape of trichomes, fruit epidermis, section through the petiole, section through the leaf blade and number of seeds.

## Introduction

FROST (1926) divided chimeras according to their origin, which has no connection with the relationship of the components. He recognized autogenic chimeras arising from somatic mutations, and synthetic chimeras in which an artificial measure, specifically grafting, plays a part in the joining of two or more genotypes in the growth apex. The whole question of chimeras is closely connected with the anatomy of the vegetative cone, in which the number of cell layers which are genetically independent of each other determines the formation of monochlamydeous, dichlamydeous etc. chimeras. A considerable volume of work is, therefore, directed to these questions (NOACK 1922, SCHWARZ 1927, LANGE 1927, CLOWES 1957).

WINKLER (1935, 1938) distinguished three layers in the vegetative cone of periclinal chimeras: ectosoma, mesosoma and endosoma, which do not correspond in their delimitation with the embryonic layers of the tunica-corpus. On this basis he distinguishes:

1. ectochimeras (*Solanum koelreuterianum*, *S. gaertnerianum*, *S. tubingenense*, *S. proteus*, etc.);
2. mesochimeras, in which a layer of one component is inserted between layers of the second component (e.g. Winkler's *S. mendelianum*, or chimeras described by DERMEN 1947, 1951 in apple and cranberry).

The historical graft chimeras (bizarre, *Laburnocytisus adami*, *Crataegomispili*) aroused interest in their time, but the main attention was paid to them once more following WINKLER and BAUR's fundamental theoretical work.

Since the practical application of graft chimeras met with no success (SAHLI 1913, 1916, KLEBAHN 1918), research work with them continued to be directed mainly to theoretical questions. Attention was paid to the mutual influence between the components and the effect on the sexual progeny. The question is whether the two kinds of plant tissue which form one morphological unit lose their genetic individuality and their genotypes acquire characters of the new phenotype, or whether their genetic independence remains unchanged. The oldest known graft chimeras were already considered as graft hybrids (e.g. Darwin in *L. adami*). Winkler himself was convinced at first that his experiments had demonstrated the possibility of forming hybrids in a vegetative manner, i. e. by grafting. There is, however, a fundamental difference between his conception of vegetative hybridization as the fusion of the nuclei of two somatic cells which had been transected during grafting, resulting in the formation of some sort of vegetative zygote (as he assumed with his "burdons"), and the conception of vegetative hybridization as a consequence of mutual genetic influence by assimilates passing from one component to the other (GLUSHCHENKO 1948a, b, LYSENKO 1952, BAZAVLUK 1952, GLAVINICH 1949, 1957, AVAKYAN 1957).

In general it can be said, however, that the findings of the authors do not differ so much as regards facts as in the construction put upon them. This may be due either to different connotations of the terms used (e. g. hybrid, hybridization, heredity), or to failure by the author to exhaust all possible means of arriving at an explanation.

## Material and Methods

The tomato *Lycopersicon esculentum* MILL. cv. Immun and *Solanum nigrum* L. with yellowish-green fruits were used in the experiments. Winkler's method was used for the production of chimeras. This depends on the mutual grafting of both components, with subsequent decapitation at the point of fusion and the creation of favourable conditions for callus formation. Cleft grafting was more successful. Following cutting at the point of fusion, the growth apices from the leaf axils were removed in order to prevent weakening of the growth of adventitious buds growing from the callus formed at the cut surface. The plants require to be kept in a moist atmosphere so that the cut surfaces do not dry up. Cuttings were made from the chimeras and planted out independently. The seeds from the chimeras were germinated on filter paper in Petri dishes and at the cotyledon stage the seedlings were transplanted to flower pots.

Measurement of stomata was carried out by the microrelief impression method (WOLF 1954) with acetone varnish. The stomata were measured on the under surfaces of leaves which had finished their growth.

## Results

Two types of chimeras were obtained—*Solanum koelreuterianum* and *S. gaertnerianum*. Winkler's original binomial nomenclature has been used in view of its simplicity, although it cannot be fully justified from the taxonomical point of view.

*Solanum koelreuterianum* originated in several specimens. It was already known from the work of other authors that this monochlamydeous chimera with nightshade epidermis is notable for its high degree of sterility. Not a single seed was obtained from the present material. The flowers fall either as buds or soon after opening. Only exceptionally was a parthenocarpic fruit formed.

*Solanum gaertnerianum* developed in 1956 from the grafting of nightshade on tomato. In the majority of its characteristics it tends to resemble nightshade. However, its type of growth is different, the shoots do not stop growth and the plants reach a much greater height (over 1 m).

The flowers are white, mostly five-petalled and in size and form the flower organs resemble nightshade. Other flowers have a multiplied number of petals, in other cases the petals develop irregularly (some are deformed, others normal). There are interesting cases of flowers with petals and stamens very much reduced in size but multiple in number, surrounding an enlarged ovary with a large style, which survives for a long time.

This plant produced no fruits in the year of grafting, in the second year it produced some in the autumn and the greatest number of fruits were formed by clones in spring 1958. The shape of the fruit shows a transition from flattened to oval and finally to very elongated fruits. The elongated forms never contained any seeds.

The shapes of the leaves are very varied. The margins are usually entire, lobed or with an indication of pinnation. The leaf blade is often asymmetric. The leaf bases pass from heart shape to an elongated junction with the petiole. Very long petioles are striking, as is the helmet-like curvature of the leaf blade with a bulge on the upper surface.

The chimera seeds were sown from fruits which ripened in the autumn of 1957. Forty plants were grown from them, all pure *Solanum nigrum* with yellowish-green fruits like the original component of the chimera.

#### Comparison of characteristics of the chimera, its seed progeny and control plants.

Size of stomata. Stomata were measured in all cases on the same part of the leaf blade, in the centre, but not immediately at the main vein. The measurement showed:

1. *S. nigrum* has larger stomata than *L. esculentum*.
2. The chimera *S. gaertnerianum* does not differ as regards size of stomata from its seed progeny.
3. The seed progeny of chimeras do not differ as regards size of stomata from the control.

Shape of stomata. No significant differences in shape of stomata were found between the different forms examined. Those of tomato (cv. Immun), nightshade, *S. gaertnerianum* and its seed progeny are oval on the lower leaf surfaces, only on the upper surface in tomato some have a single protrusion at one or both ends. This character was not observed in the chimera, but an analogous form was met with in control nightshade plants and the seed progeny. In these cases, however, it is possible by focussing the microscope on the lower part of stoma to observe that the phenomenon is due to a fold of the neighbouring epidermal cell. In tomato there may be an actual thickening of the cell wall at the point of contact and fusion of both guard cells.

Trichomes. In all forms there are basically two kinds, glandular and protective.

Protective trichomes of tomato are usually formed of three cells, of which the apical narrows to the point, while in nightshade they are in most cases club-shaped. The species to which the epidermis of a chimera belongs can be easily recognised by means of these trichomes. Occasionally pointed trichomes of the tomato type were found on some leaves of chimeras, their seed progeny and of control nightshade plants.

Glandular trichomes have a one-celled, rarely two-celled stem on which rests a multicellular head. In tomato this head is made up of one small cell with four round cells on it. In nightshade these short trichomes are varied in form, but trichomes with four-celled heads were not observed on leaves of nightshade or chimeras.

Epidermis of fruits. There are considerable differences between the two parent species. Tomato fruits have an epidermis with very thick-walled cells, nearly square in shape. In nightshade the cells are larger, more rectangular, but with cell walls of about a quarter of the thickness of those in tomato. In the *S. gaertnerianum* chimera (diectochimera) and its seed progeny the cell walls of the fruit epidermis were found to differ in no way from those of the nightshade control.

Section of the petiole. The base of the petiole is similar in all the plants examined. There are either winged petioles (nightshade) or there are indica-

tions of wingedness as in tomato. The vascular bundles are bicollateral in both species, the wood in nightshade falls into three parts, in tomato and chimeras it is divided into many parts, separated by rows of parenchymatous cells. The seed progeny of the chimeras did not differ as regards this characteristic from the control nightshade.

Leaf section. The two parent species differ from each other as regards the proportions of palisade and spongy parenchyma. While palisade cells form nearly one-half of the thickness of the leaf in nightshade, they form only about one-third in tomato leaves. In the chimera *S. gaertnerianum*, where the palisade tissue is formed from the outer component and tomato forms only a smaller part of the leaf blade, the leaf section resembles nightshade in the proportions of its mesophyll. The seed progeny also resemble the control plants.

The effect on the fertility of progeny was followed according to the number of seeds in the berries. In ten plants taken at random from the seed progeny of the chimera, seeds were counted in ten berries from each plant, i.e. in a total of 100 berries. Similarly 100 berries from ten plants were examined in the control. In the seed progeny the average number of seeds in one berry equals  $\bar{x} \pm 3 \cdot s_{\bar{x}} = 30.25 \pm 3 \cdot 1.15$ . For control nightshade plants with yellowish-green fruits  $\bar{x} \pm 3 \cdot s_{\bar{x}} = 33.00 \pm 3 \cdot 1.14$ .

$t_{198} = 1.62$ . For  $P = 0.01$   $t_{200} = 2.60$  and for  $P = 0.05$   $t_{200} = 1.98$ . As the results show, the seed progeny are not influenced in their fertility and completely agree with their controls as regards this character.

## Discussion

A great deal has already been written about the so-called vegetative hybridization from various points of view. RYZHKOV (1947) wrote on the subject of including chimeras among vegetative hybrids: "If the term hybrid is used in the exact sense, i.e. if we mean the product of fusion of two cells, then this is in no case a hybrid".

This criterium could be fulfilled only by the so-called burdon, the existence of which has not yet been demonstrated.

If we take into consideration that in more recent genetic definitions a hybrid is identified with a heterozygote, then the condition remains that the hybrid nature of an organism, if it is fertile, must evince itself in some form in later generations.

According to T. D. Lysenko vegetative hybridization would be a relatively easy matter. "It is necessary to force the scion to build up its body completely from nutrients which are supplied by the roots of the stock and from plastic substances produced by the roots of the stock and from plastic substances produced by assimilation by the stock's leaves. In such cases vegetative hybridization will always be completely successful." (Lysenko, p. 239.)

During the formation of the chimera only an insignificant wedge of the second component without assimilatory surface remains after decapitation, from which in conjunction with the first component the chimera shoot de-

velops, which in fact builds up the whole of its body from nutrients and "plastic substances" supplied by the stock. In the experiment described above the supplier was represented by the tomato, and nevertheless neither in the micromorphological characters of the seed progeny nor in the nightshade tissues of the chimera itself was there evidence of any specific influence.

The group of authors already mentioned explain this as being due to developing zygotes in the flowers of chimeras which are able to choose the nutrients of only one genetic norm in agreement with Michurin's teaching on the impossibility of mixing specific characters. (GLUSHCHENKO 1948a, b, BAZAVLUK 1952, AVAKYAN 1957.)

Long before this theory was evolved it was known that in the great majority of cases gametes are built up from the subepidermal cell layers. It was not, therefore, surprising that the progeny of monochlamydeous chimeras are identical with the inner component, the dichlamydeous with the outer. There was thus no demand for a theory which would endow certain cells with such exceptional abilities. Only following the assumption that the transfer of assimilates from one component to the other implies the passing on of hereditary characters, did it become necessary to explain why these characters are never expressed in the sexual progeny. This is therefore a speculative rather than an experimentally founded theory.

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Address: V. Fučík, Prom. Biol., Ústav organické chemie a biochemie ČSAV Praha 6, Na evičišti 2.

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### Семенное потомство пасленовых химер

Владимир Фучик

Отделение генетики Естественного факультета Карлова Университета, Прага

#### Резюме

В статье описывается получение химер между томатом и пасленом (*Solanum koelerianum* и *S. gaertnerianum*).

По анатомическим и микроморфологическим признакам не удалось у *S. gaertnerianum* обнаружить какого-либо специфического влияния между тканями.

Семенное потомство химеры *S. gaertnerianum* по всем признакам сходно с растениями паслена.

Исследовались следующие признаки: величина устьиц, форма устьиц, форма трихом, эпидермис плодов, разрез через черешок, разрез через листовую пластинку и количество семян.

## Respiratory Pathways in Gibberellin-Treated Wheat The Effect of Fluoride on the Respiration Rate

JIRÍ LUŠTINEC, JAN KREKULE and VĚRA POKORNÁ

Department of Plant Physiology, Institute of Biology, Czechoslovak Academy of Sciences, Praha

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### Souhrn

1. Bylo zjištěno, že fluorid inhibuje silněji dýchání listů pšenice pěstované v roztoku kyseliny gibberelové než dýchání listů kontrolních rostlin pěstovaných ve vodě v průběhu celého vývoje 1. listu.

2. Dýchání je silně bržděno i u starých listů pokusných rostlin, zatím co dýchání stejně starých listů rostlin kontrolních fluorid stimuluje o 10—100 %.

3. Předpokládá se, že posun rovnováhy dýchacích cest ke glykolyse, na nějž možno usuzovat z uvedených pokusů, souvisí s růstovým účinkem kyseliny gibberelové.

### Summary

1. It was found that fluoride has a more pronounced inhibitory effect on the respiration of wheat leaves in plants grown in a solution of gibberellic acid than on that of control plants grown in water, during the entire development of the first leaf.

2. Respiration is markedly inhibited even in old leaves of gibberellin-treated plants while the respiration of equally old control plants is stimulated by fluoride by 10—100%.

3. It is assumed that the shift in the equilibrium of respiratory pathways to glycolysis, as is indicated by respiration experiments, is associated with the growth effect of gibberellic acid.

## Introduction

As was found by GIBBS and BEEVERS (1955) with the aid of specifically labelled glucose, the glycolytic pathway predominates in young and non-differentiated tissues whereas in older tissues respiration proceeds mostly *via* the pentose cycle. In this connection experiments were carried out (GIBBS and BEEVERS 1955; SHAW, SAMBORSKI and OAKS 1958) as to the effect of indoleacetic acid on the equilibrium between glycolysis and the glucose monophosphate pathway and it was found that at low concentrations when growth is stimulated and oxygen consumption increased there were no detectable changes in this equilibrium. The last-named authors found, however, that at somewhat higher concentrations of indoleacetic acid the activity of the glucose monophosphate pathway is increased. A marked shift of the equilibrium of respiratory pathways to the pentose cycle was also achieved by HUMPHREYS and DUGGER (1957) who used high concentrations of this compound.

It was stated in a previous communication (LUŠTINEC and KREKULE 1959) that fluoride markedly inhibited the respiration of leaves of wheat grown in a solution of gibberellic acid which led the authors to the conclusion that gibberellic acid could activate the glycolytic pathway of respiration. It is the aim of the present paper to demonstrate the relationship between the effect of gibberellic acid and the age of the leaf.

## Material and Methods

Leaf blades of the first leaves of wheat seedlings (var. *Hodonínská holice*) were used for the experiments. The plants were germinated in distilled water and then grown in an illuminated box on a 12-hour day régime (illumination with 25 W fluorescent tubes) at 20–24° C in Petri dishes containing either distilled water or a solution of gibberellic acid (20 mg./l.).

Respiration experiments: At the end of the light period the plants were cut off about 0.5 cm. above the seed and submerged with their lower part in water or in  $3 \times 10^{-2}$  M-NaF. They were kept in these liquids in the dark at 25° C for 10 hours. Respiration was measured in the dark at 30° C by the conventional Warburg manometric method. Each flask contained 5 to 10 blades of the first leaf.

## Results and Discussion

The effect of gibberellic acid on the growth and respiration of wheat leaves is apparent at the very beginning of the development of the leaf still concealed in the coleoptile. During development, the differences in leaf length and in their dry weight, increase to reach maximum values between the eighth and ninth day after germination (submersion of seeds in water) (Figs. 1, 2). The intensity of leaf respiration of plants, both experimental and control, decreases in parallel from the initial value of  $Q_0$ , 6–7 to about 2–3 on the 11th day of leaf development. At the same time, the respiratory

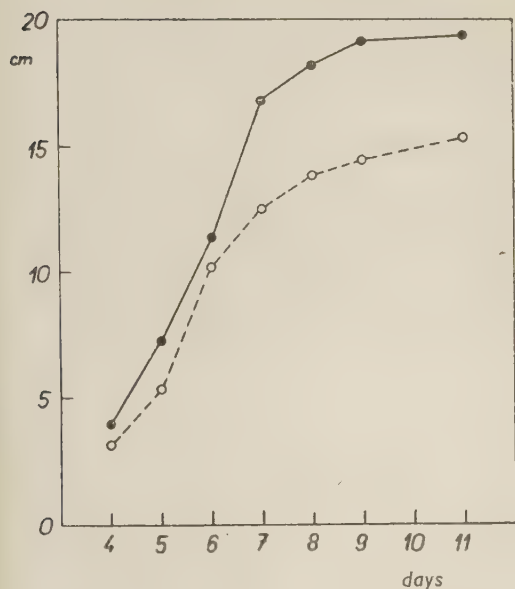


Fig. 1. The effect of gibberellic acid on the growth of wheat leaves. Ordinate: leaf length in cm. Abscissa: time after germination of seeds. Each value represents an average of measurements of 10 leaves. Broken line — control; full line — gibberellic acid.

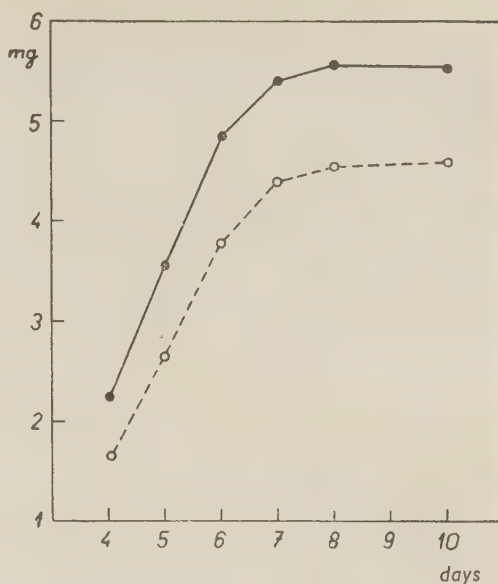
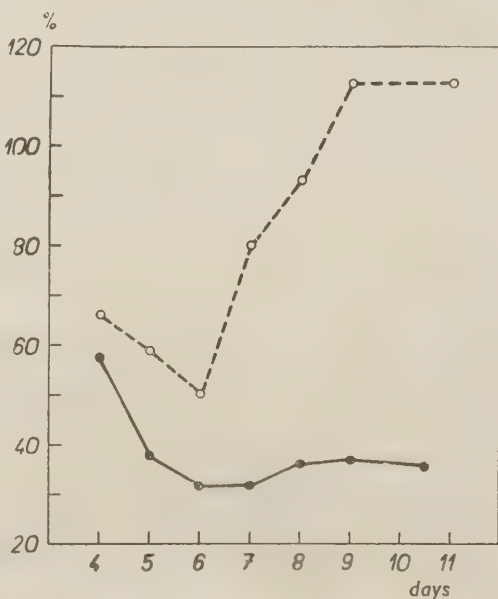


Fig. 2. Production of leaf dry matter of control and gibberellin-treated plants. Ordinate: mg dry matter per 1 leaf. Abscissa: time after germination of seeds. Each value represents an average of five samples, each containing 10 leaves. Broken line — control; full line — gibberellic acid.

Fig. 3. Inhibition of leaf respiration by fluoride expressed as per cent respiration of uninhibited leaves. Ordinate: per cent inhibition. Abscissa: time after germination of seeds. Broken line — control; full line — gibberellic acid.

intensity of experimental plants is 10–50% higher than that of controls. Gibberellic acid brings about an increase in the inhibitory effect of fluoride on respiration at an early stage of development. Before the sixth day the degree of inhibition increases gradually both in the experimental and control leaves, then a marked decrease in the inhibitory effect in the controls takes place while the degree of inhibition of experimental leaves does not change significantly. Fluoride markedly inhibits respiration even in old leaves of gibberellin-treated plants whereas the respiration of identically old



leaves of control plants is even stimulated by 10–100% (Fig. 3). Increase in respiration due to fluoride treatment of leaf disks has also been estimated by McNULTY and IRVING (1959).

On the basis of the above findings it may be assumed that gibberellic acid activates glycolysis during the early stages of leaf development. It is therefore possible that the shift of the equilibrium of respiratory pathways toward glycolysis is directly connected with its growth effect. The increase in respiratory intensity of experimental plants can be due to an activation of the glycolytic pathway (LUŠTINEC and KREKULE 1959) provided that the terminal oxidases or other metabolic systems do not represent a limiting factor of this process.

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*Address:* Jiří Luštinec, Prom. biol., Ing. Jan Krekule, Dr. Věra Pokorná, Biologický ústav ČSAV, Praha-Dejvice, Na cvičišti 2.

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### Дыхательные пути гиббереллированной пшеницы Влияние фтористого натрия на скорость дыхания

Иржи Луштинац, Ян Крекуле и Вера Покорна

Отделение физиологии растений Биологического института Чехословацкой академии наук, Прага

#### Резюме

1) Установлено, что фтористый натрий ингибирует дыхание листьев пшеницы, выращенной в растворе гиббереллиновой кислоты сильнее чем дыхание контрольных растений, выращенных в воде в течение всего развития 1-го листа.

2) Сильно заторможено и дыхание старых листьев подопытных растений, между тем как дыхание листьев одинакового возраста у контрольных растений стимулировано фтористым натрием на 10–100%.

3) Высказывается предположение, что смещение равновесия дыхательных путей в направлении к гликолизу, о чем можно судить по результатам дыхательных опытов, связано с действием гиббереллиновой кислоты на рост.

## Etude microcalorimétrique de l'accélération de la germination des graines, traitées par les ultra-sons

† GEORGE OBOLENSKY

Fac. Sci. - Paris et Fac. Sci. Marseille

*Accepté le 18 Janvier 1960*

### Souhrn

Semena ječmene odrůdy „Freja“ a „Rike“ byla vystavena po dobu 5—25 min. různým frekvencím ultrazvuku (30, 80, 570, 720, 960 kHz) o intenzitě 2—5 W/cm<sup>2</sup>. Pomocí mikrokolorimetrických měření byla sledována kinetika thermogenese u pokusných i kontrolních semen. Bylo zjištěno, že doba trvání fysikálně chemické thermogenese (příjem vody, botnání koloidů, atd.) je podstatně snížena v případě pokusných semen. Dále nastává rychlý přechod z fáze fysikálně chemické do fáze biologické thermogenese, která dokonce může převyšovat fázi fysikálně chemickou.

Působení ultrazvuku v závislosti na době působení a na frekvenci tedy vyvolává všeobecné urychlení metabolických procesů, projevující se urychlením klíčení. Tento jev je irreversibilní a uplatňuje se i několik týdnů a měsíců po ovlivnění.

### Résumé

On a traité des graines d'orge à différentes fréquences ultrasonores (30, 80, 570, 720, 960 kHz) avec des puissances de 2 à 5 W/cm<sup>2</sup>, de 5 à 25 minutes, en vue d'étudier le mécanisme d'action des ultra-sons sur le processus de la germination.

A cet effet, nous avons effectué des enregistrements microcalorimétriques au laboratoire de Monsieur le Professeur E. CALVET à la Faculté des Sciences de Marseille, en vue de comparer les courbes représentant la cinétique de la thermogénèse dans le cas des graines traitées ou non traitées par les ultra-sons.

† GEORGE OBOLENSKY, mort à Paris le 2 mai 1960.

Les courbes montrent que la durée de la thermogénèse physico-chimique caractérisée par la première pointe exothermique est diminuée dans le cas des graines traitées et que le phénomène passe alors rapidement de la phase physico-chimique à la phase de thermogénèse biologique, la deuxième phase pouvant même se superposer à la phase physico-chimique.

Ainsi le traitement provoque une accélération de la germination et modifie favorablement la durée de celle-ci (thermogénèse biologique plus active). Le phénomène d'accélération de la germination se manifestant de la même manière sur des graines dont la thermogénèse fut étudiée plusieurs semaines et plusieurs mois après le traitement aux ultra-sons, cela montre que cette action est irréversible et correspond principalement à une modification de la perméabilité des membranes. Il est cependant possible qu'il vienne s'y associer des actions dépolymérisantes (PRAT, CALVET 1945) (dépolymérisation de l'amidon en dextrine) qui expliqueraient, mieux que la seule absorption d'eau, la prolongation dans le temps de l'accélération de la croissance (diminution de la phase endothermique et accélération de la phase exothermique).

Ainsi la thermogénèse des graines traitées aux ultra-sons, grâce à la grande précision de la méthode microcalorimétrique, nous montre que la pénétration ultérieure de l'eau dans les graines, ainsi que les processus enzymologiques produisant la thermogénèse biologique, sont nettement accélérés par les ultra-sons.

### Introduction

Nous avons antérieurement montré que l'accélération de la croissance des graines d'orge soumises à l'action des ultra-sons provoque une accélération de la germination, en augmentant le pouvoir germinatif. Avec  $P^{32}$  on a observé que l'accélération de la germination reste acquise dans la plantule pendant 10 jours et que le métabolisme de la plantule issue des graines traitées était plus actif que celui des témoins. Toutefois, il restait encore à démontrer et à confirmer, par une méthode physiologique rigoureuse, que les différences constatées entre les graines traitées et les graines non traitées reposaient bien sur des processus métaboliques.

C'est pourquoi nous avons envisagé de mesurer directement les quantités de chaleur dégagées par l'augmentation de l'intensité respiratoire due à la germination. À cet effet, nous avons fait appel à la méthode microcalorimétrique du Professeur E. CALVET (1954) qui permet d'enregistrer fidèlement et avec une grande précision, en fonction de temps, de très faibles débits thermiques et de comparer ainsi les courbes de cinétique et de thermogénèse des graines traitées et de celles non traitées aux ultra-sons.

### Appareillage et mode opératoire

Des graines d'orge des variétés „Rika“ ou „Freja“ ont été traitées aux ultra-sons dans des cuves à eau avec générateur à quartz ou à magnétostriction, aux diverses fréquences contrôlées de 30, 80, 570, 720 et 960 kHz; les puissances acoustiques développées variaient de 2 à 5 W/cm<sup>2</sup>.

Les graines étaient traitées dans un courant d'eau passant par un manchon perforé en plexi-glass qui les maintenait dans l'axe du faisceau ultrasonore, au-dessus du cristal ou de la surface émettrice. Le courant d'eau agitait les graines de façon à les faire passer régulièrement par le faisceau ultrasonore; en outre, ce procédé permettait de maintenir la température de l'eau à une valeur constante: de 16 à 17° C.

Les traitements ont été effectués chaque fois sur 200 graines, de manière à pouvoir prélever des échantillons moyens représentatifs destinés aux essais de thermogénèse.

L'étude sur la thermogénèse de la germination de l'orge traitée aux ultra-sons fut réalisée suivant la méthode de PRAT et CALVET- (1944, 1945) et PRAT (1951) en utilisant 1 gr de graines d'orge traitée ou non aux ultra-sons et additionnée de 1 cm<sup>3</sup> d'eau distillée. Le dispositif utilisé est représenté à la figure 3. Les graines d'orge sont placées dans la cellule laboratoire de l'appareil et l'eau dans une ampoule de verre mince fermée par une pointe effilée. La mise en contact des graines et de l'eau est obtenue en brisant à l'aide d'une tige poussoir manoeuvrée de l'extérieur, la pointe de l'ampoule. Une poire de caoutchouc terminant la tige permet de vider complètement l'ampoule.

On opérait toujours à la température de 25° C, car ce facteur agit sur la thermogénèse germinative en l'accélérant (OBOLENSKY 1953, OBOLENSKY 1957, PRAT, CALVET 1944).

La température de 25° C a donc été choisie pour éviter un trop grand accroissement de la chaleur dégagée. Enfin, on employait de l'eau distillée pour éviter des déformations des courbes thermogénétiques dues à l'action des substances dissoutes dans l'eau (CHOUARD 1949).

### Résultats expérimentaux

L'utilisation du microcalorimètre E. CALVET nous a permis de montrer, en appliquant une méthode physiologique rigoureuse, que les différences observées entre les graines traitées et les graines non traitées reposaient bien sur des processus métaboliques. La comparaison des courbes représentant la thermogénèse germinative des graines traitées et non traitées par les ultra-sons met clairement en évidence l'action activante de ces derniers.

Par intégration des courbes après 10, 20 et 30 heures d'enregistrement, on a pu suivre la variation en fonction du temps, de la quantité de chaleur dégagée au cours de la germination.

La simple comparaison des quantités de chaleur dégagées en opérant sur des graines traitées et non traitées montre que les ultra-sons produisent une accélération très nette de la germination. Il semble que dans nos conditions opératoires et dans les limites des puissances acoustiques utilisées par nous, cette action ne soit pas liée à la longueur d'onde, mais dépende de la puissance.

Dans certains cas, on a observé que les courbes de thermogénèse germinative de graines traitées et non traitées se recoupent ou se confondent, ce qui confirmerait les résultats de GLAUSER (1951) et de SASAGAWA (1939) relatifs à l'influence de la puissance ultrasonore appliquée et à la longueur d'onde, lesquelles donnent des effets spécifiques avec des seuils comme le font d'autres agents physiques ou chimiques.

D'autre part, le taux d'humidité des graines mises en oeuvre exerçant une grande influence sur l'allure de la thermogénèse physico-chimique et de la thermogénèse biologique, il était important de ramener par dessiccation les graines ultrasonnées à leur teneur en eau initiale (OBOLENSKY 1957). Cette précaution est d'ailleurs indispensable pour permettre de réaliser au début de l'expérience la stabilisation du zéro du microcalorimètre et cela en raison du dégagement de chaleur produit par la germination prématurée

des graines humides. En outre, ces graines déjà humides n'absorbant que très peu d'eau au cours de l'essai microcalorimétrique proprement dit, la phase physico-chimique de la thermogénèse est écourtée, ce qui ne permet plus une étude correcte des divers phénomènes (adsorption d'eau, imbibition des colloïdes, dissolution, etc...) se produisant au cours de cette phase.

Un examen attentif des courbes nous montre que la partie de la thermogénèse physico-chimique correspondant à l'humectation des graines est écourtée dans le cas des graines traitées et qu'elle est suivie d'une période de latence très brève. En d'autres termes, la phase de latence devient rapidement exothermique, contrairement à ce qui se passe dans le cas des graines non traitées pour lesquelles elle demeure endothermique un certain temps. En outre, on peut remarquer que, pour tous les traitements de durées variables effectués à diverses fréquences, on observe sur les courbes un dégage-

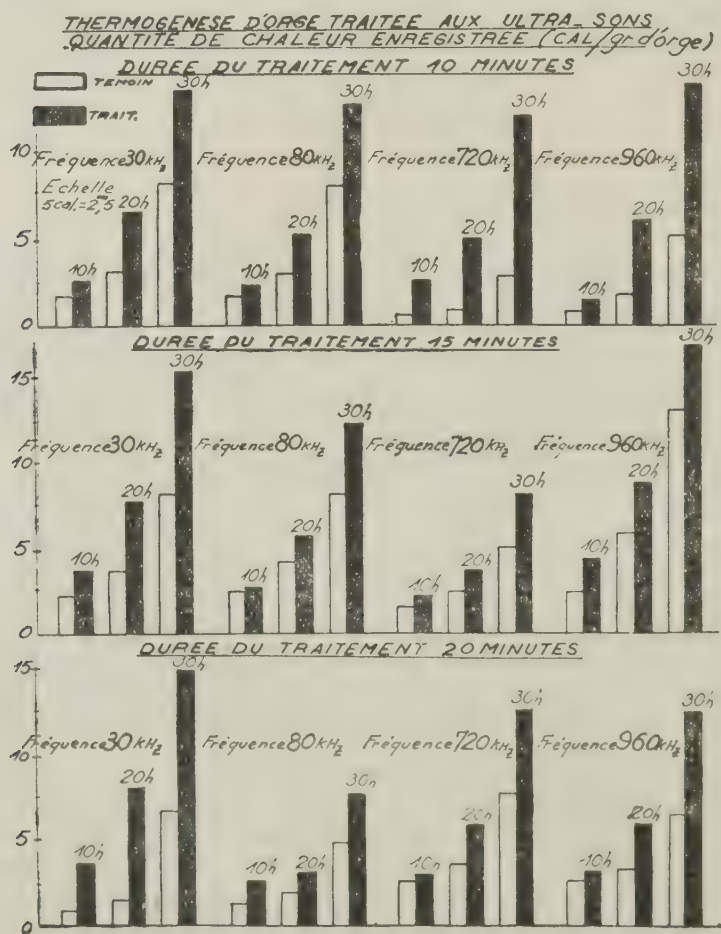
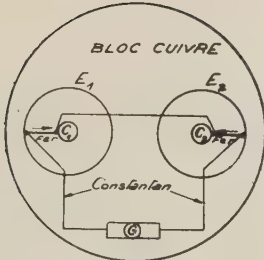


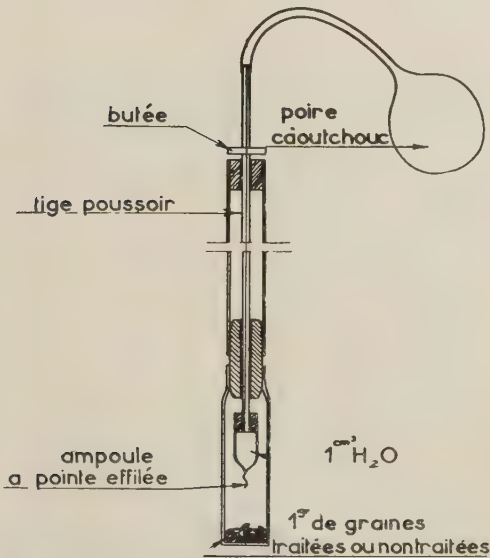
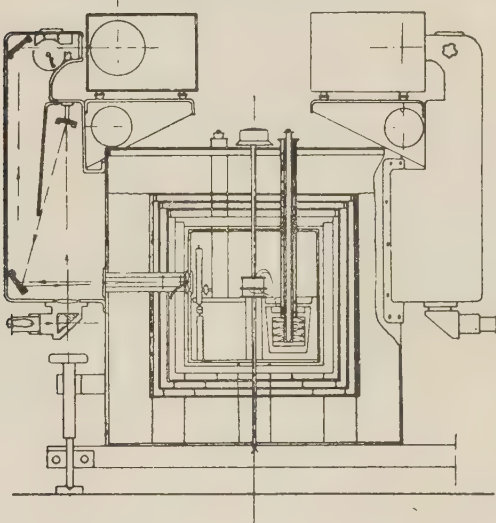
Fig. 1.

Microcalorimètre différentiel type Calvet

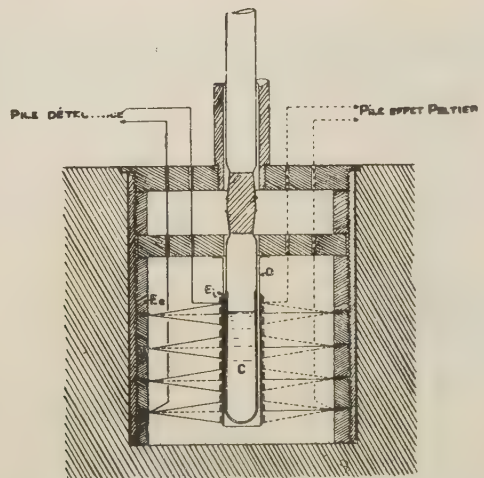


SCHEMA DU MICROCALORIMETRE DIFFERENTIEL

G - Galvanomètre  
G<sub>1</sub>-G<sub>2</sub> Cellules (Enceintes Internes)  
E<sub>1</sub>-E<sub>2</sub> Enceintes externes



DISPOSITIF SPECIAL POUR L'ETUDE DE LA THERMOGENESE DE LA GERMINATION SUIVANT LA METHODE DE M<sup>rs</sup> PRAT ET CALVET

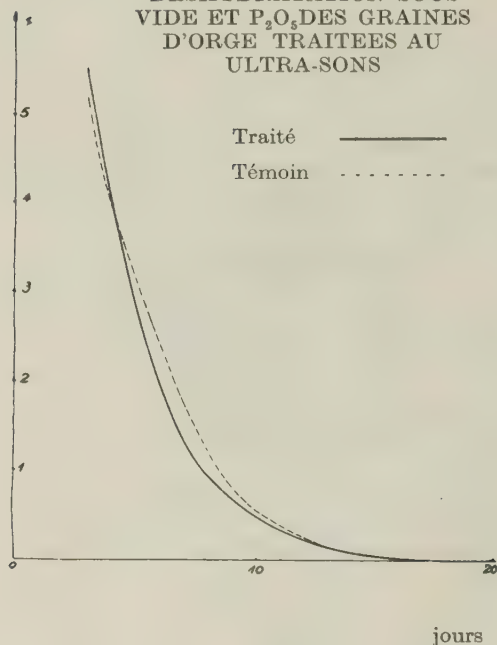


Ei - ENCEINTE INTERNE  
Ee - EXTERNE  
D - DOUILLE D'ARGENT  
C - CELLULE VERRE CUIVRE

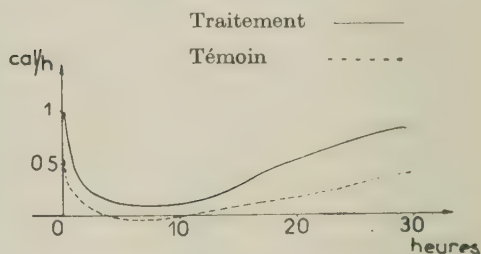
Fig. 2 - Dispositif de microcalorimétrie avec cellule laboratoire.

Fig. 1

### DESHYDRATATION SOUS VIDE ET $P_2O_5$ DES GRAINES D'ORGE TRAITEES AU ULTRA-SONS

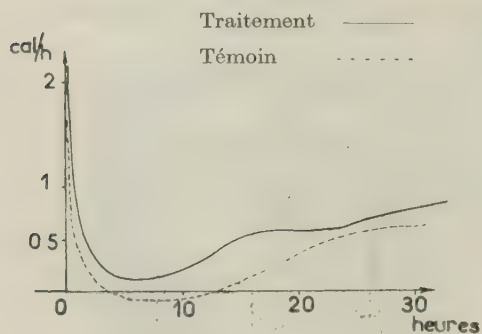


### THERMOGENESE D'ORGE « FREJA » TRAITE AUX ULTRA-SONS



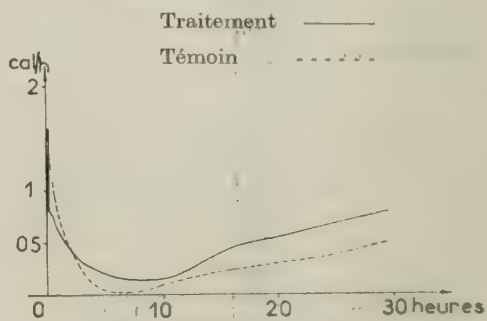
Traitement 720 KHz — 0,4 Amp. 3,5 W/cm<sup>2</sup> — 10 min. (5 semaines après traitement). 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Chaleur enregistrée en cal.: Traitement: 2,5 en 10 h; 5,05 en 20 h; 12,2 en 30 h. Témoin: 0,55 — 10 h; 0,85 en 20 h; 2,82 en 30 h.

### THERMOGENESE D'ORGE TRAITE AUX ULTRA-SONS



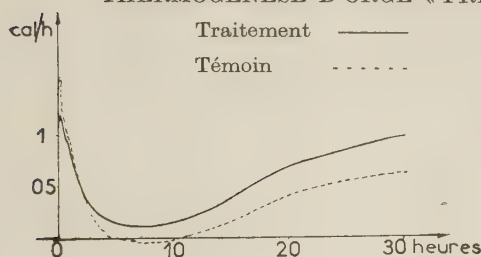
Traitement 30 KHz — 2,5 W/cm<sup>2</sup> — 20 min. Quantité de chaleur enregistrée (cal). Traitement: 3,5 cal en 10 h; 8,1 cal en 20 h; 14,9 cal en 30 h. Témoin: 0,705 cal en 10 h; 1,35 cal en 20 h; 6,65 cal en 30 h.

### THERMOGENESE D'ORGE TRAITE AUX ULTRA-SONS

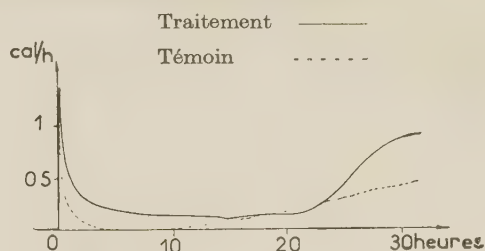


Traitement 80 KHz — 4 W/cm<sup>2</sup> — 15 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de chaleur enregistrée en cal.: Traitement: 2,5 en 10 h; 5,63 en 20 h; 12,15 en 30 h. Témoin: 2,42 — 10 h; 4,26 en 20 h; 8,10 en 30 h.

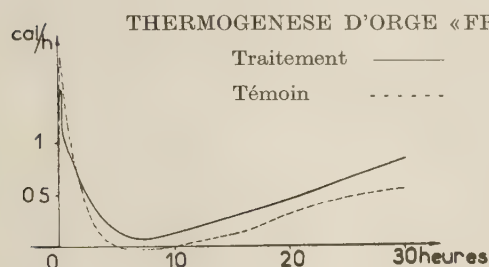
## THERMOGENESE D'ORGE « FREJA » TRAITE AUX ULTRA-SONS



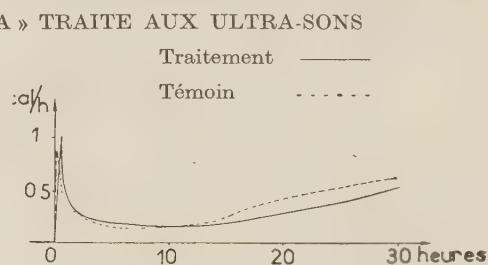
Traitement 720 KHz — 0,4 Amp. 3 W/cm<sup>2</sup> — 15 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de chaleur enregistrée en cal.: Traitement: 2,19 en 10 h; 3,55 en 20 h; 8,02 en 30 h. Témoin: 1,53 — 10 h; 2,36 — 20 h; 5,0 — 30 h.



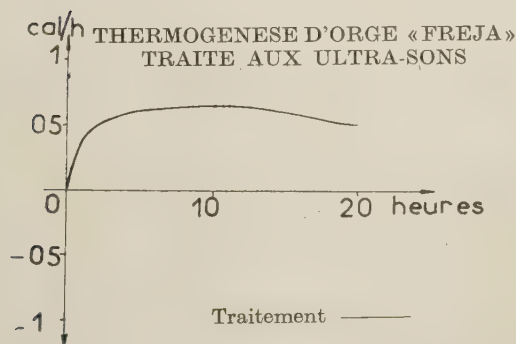
Traitement 30 KHz — 2,5 w/cm<sup>2</sup> — 10 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Brisant la pointe de l'ampoule contre les graines. Quantité de chaleur enregistrée en cal.: Traitement: 2,80 en 10 h; 6,74 en 20 h; 13,80 en 30 h. Témoin: 1,78 en 10 h; 3,18 en 20 h; 8,35 en 30 h.



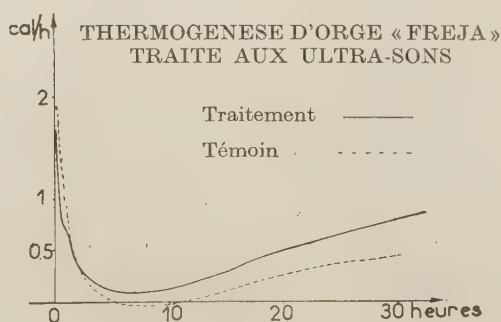
Traitement 720 KHz — 0,4 Amp. 3,5 W/cm<sup>2</sup> — 20 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de chaleur enregistrée en cal.: Traitement: 2,8 en 10 h; 5,75 en 20 h; 12,5 en 30 h. Témoin: 2,4 — 10 h; 3,3 — 20 h; 7,6 — 30 h.



Traitement 960 KHz — 0,4 Amp. — 3,5 W/cm<sup>2</sup> — 25 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de chaleur enregistrée en cal.: Traitement: 1,78 en 10 h; 3,56 en 20 h; 7,6 en 30 h. Témoin: 1,69 en 10 h; 3,76 en 20 h; 8,95 en 30 h.

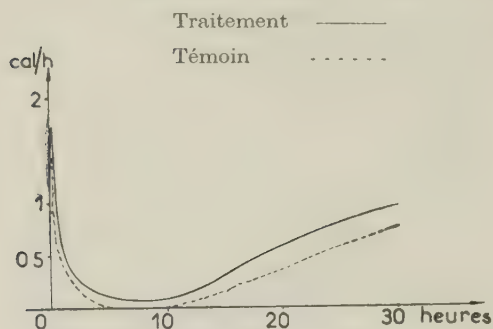


Traitement 960 KHz — 0,4 Amp — 3,5 W/cm<sup>2</sup> — 20 min. Courbes différentielles. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée (trait. et témoin). Quantité de chaleur enregistrée (cal): 5,45 pour 10 h; 11,24 pour 20 h.

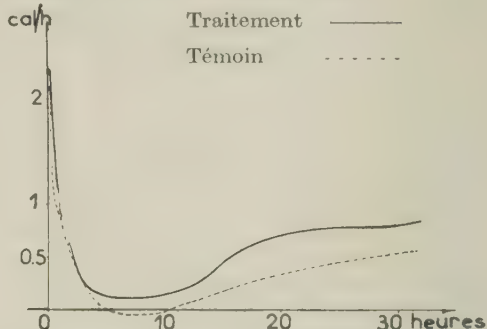


Traitement 960 KHz — 0,4 Amp — 3,5 W/cm<sup>2</sup> — 20 min. 1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Brisant la pointe de l'ampoule contre les graines. Quantité de chaleur enregistrée en cal.: Traitement: 3,02 en 10 h; 5,86 en 20 h; 12,4 en 30 h. Témoin: 2,39 en 10 h; 3,06 en 20 h; 6,30 en 30 h.

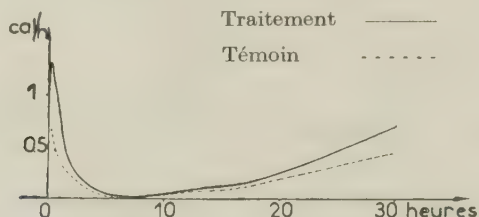
## THERMOGENESE D'ORGE « FREJA » TRAITE AUX ULTRA-SONS



Traitement 80 KHz — 4 W/cm<sup>2</sup> — 10 min.  
1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de  
chaleur enregistrée en cal.: Traitement: 2,34 en  
10 h; 5,4 en 20 h; 13,05 en 30 h. Témoin: 1,7 en  
10 h; 3,04 — 20 h; 8,1 en 30 h.



Traitement 30 KHz — 2,5 W/cm<sup>2</sup> — 15 min.  
1 g. d'orge + 1 cm<sup>3</sup> H<sub>2</sub>O distillée. Quantité  
de chaleur enregistrée. Traitement: 3,75 cal en  
10 h; 7,75 cal en 20 h; 15,6 cal en 30 h. Témoin:  
2,25 cal en 10 h; 3,72 cal en 20 h; 8,30 al en  
30 h.

THERMOGENESE D'ORGE « FREJA »  
TRAITE AUX ULTRA-SONS

Traitement 80 KHz — 4 W/cm<sup>2</sup> — 20 min.  
1 g. d'orge + 1 cc H<sub>2</sub>O distillée. Quantité de  
chaleur enregistrée en cal.: Traitement: 2,42 en  
10 h; 3,02 en 20 h; 7,50 en 30 h. Témoin: 1,12 en  
10 h; 1,82 en 20 h; 4,88 en 30 h.

ment de chaleur plus important, se produisant de 15 à 16 heures après le début de l'expérience microcalorimétrique. Ce phénomène peut être attribué à un brusque dégagement de chaleur des graines.

Dans le cas des graines ultrasonnées 15 minutes à la fréquence de 720 kHz — 3 W/cm<sup>2</sup> ce brusque dégagement de chaleur ne s'est produit qu'au bout de 22 heures, le retard pouvant être attribué au fait que, suivant la durée du traitement, chaque fréquence pourrait avoir une action spécifique.

Les courbes des figures 6 et 7 n'ont enregistré qu'une thermogénèse physico-chimique d'importance très réduite, en raison du fait que l'expérience avait été effectuée sur des graines demeurées humides après le traitement. Toutefois, ici encore, le développement de la germination est beaucoup plus intense pour les graines traitées que pour les graines non traitées.

Enfin, les données relatives aux quantités de chaleur dégagées (exprimées en calories) rassemblées dans le tableau suivant, mettent en évidence, de façon indiscutable, l'effet stimulant des ultra-sons.

## THERMOGENESE D'ORGE TRAITÉ AUX ULTRA-SONS

1 g. d'orge + 1 cm<sup>3</sup> d'eau distillée

| Fréquence<br>(utilisée)                      | Temps du<br>traitement<br>min. | 10 h. | Quantité de chaleur enregistrée (cal.) en: |           |        |       | 30 h.    |
|----------------------------------------------|--------------------------------|-------|--------------------------------------------|-----------|--------|-------|----------|
|                                              |                                |       | Traitement                                 |           | Témoin |       |          |
|                                              |                                |       | 20 h.                                      | 30 h.     | 10 h.  | 20 h. |          |
| 960 kHz<br>5 W/cm <sup>2</sup>               | 15                             | 6,70  | 14,48                                      | —         | 2,44   | 4,12  | —        |
| 960 kHz<br>0,4 amp.<br>3,5 W/cm <sup>2</sup> | 5                              | 1,30  | 5,43                                       | —         | —      | 1,42  | 3,53 (1) |
|                                              | 10                             | 1,39  | 6,08                                       | —         | 0,66   | 1,68  | —        |
|                                              | 15                             | 4,30  | 8,80                                       | 16,70     | 2,42   | 5,90  | 13,05    |
|                                              | 20                             | 5,95  | 11,24                                      | 13,50 (2) | —      | —     | —        |
|                                              | 25                             | 1,78  | 3,56                                       | 7,60      | 1,69   | 3,76  | 8,95     |
| 720 kHz<br>0,4 amp.<br>3 W/cm <sup>2</sup>   | 10                             | 2,50  | 5,05                                       | 12,12     | 0,55   | 0,85  | 2,82     |
|                                              | 15                             | 2,19  | 3,55                                       | 8,02      | 1,53   | 2,36  | 5,00     |
|                                              | 20                             | 2,80  | 5,75                                       | 12,50     | 2,40   | 3,38  | 7,60     |
| 80 kHz<br>4 W/cm <sup>2</sup>                | 10                             | 2,34  | 5,4                                        | 13,05     | 1,70   | 3,04  | 8,10     |
|                                              | 15                             | 2,50  | 5,63                                       | 12,15     | 2,42   | 4,26  | 8,10     |
|                                              | 20                             | 2,42  | 3,02                                       | 7,50      | 1,12   | 1,82  | 4,80     |
| 30 kHz<br>2,5 W/cm <sup>2</sup>              | 10                             | 1,58  | 3,20                                       | 9,55      | 1,78   | 3,18  | 8,35     |
|                                              | 15                             | 3,75  | 7,75                                       | 15,60     | 2,85   | 3,72  | 8,30     |
|                                              | 20                             | 3,50  | 8,10                                       | 14,90     | 0,71   | 1,35  | 6,65     |

(1) 3,53 pour 27 h.

(2) 13,50 pour 26 h. (courbe différentielle)

### Discussion et conclusion

Les résultats obtenus confirment les études antérieurement entreprises à l'aide de diverses méthodes et notamment de celles basées sur le pourcentage de germination, sa vitesse et la teneur en eau. Nos travaux avec le  $P^{32}$  ont montré que l'accélération de la croissance des graines d'orge soumises aux ultra-sons paraît liée, au moins en partie, à une modification irréversible de la perméabilité des téguments de la graine, ce qui est en accord avec ce que l'on sait des actions physiques des ultra-sons sur les potentiels des membranes (BUSNEL, OBOLENSKY 1954, 1955). Cette activation peut être ralentie et la mort du germe pourrait même s'ensuivre, si l'on prolongeait la durée de l'application. En d'autres termes, les ondes ultra-sonores produiraient, par une application de trop longue durée, une destruction traumatique de la structure cellulaire (ASCHE 1951, PROUDHOMME 1949).

C'est le protoplasma cellulaire lui-même qui est influencé par les ultra-sons, des points de vue structural et fonctionnel.

Nous nous bornons ici à rappeler la théorie moderne portant sur la structure moléculaire de la cellule comme elle apparaît d'après les recherches de polarisation optique du zoologue W. J. SCHMIDT (1940) ainsi que du Prof. Frey-Wyssling. Selon lui, on trouverait, dans le protoplasma des molécules filiformes formant une grille à trois dimensions et présentant dans leur structure micellaire une sorte de squelette submicroscopique du protoplasma. Tous les autres éléments cellulaires se trouvent placés dans cette grille.

### ANALYSE DE VARIANCE

| Source de Variation                                                  | D. F.* | S. S.*  | M. S.* | F       |
|----------------------------------------------------------------------|--------|---------|--------|---------|
| Total                                                                | 71     | 1226,34 |        |         |
| F. Fréquences                                                        | 3      | 45,30   | 15,10  | < 1     |
| H. Heures (integrations<br>des courbes après 10,<br>20 et 30 heures) | 2      | 743,58  | 371,79 | 10,66** |
| R. Temps du traitement                                               | 2      | 20,14   | 10,07  | < 1     |
| Tus — Traitement<br>aux U. S.                                        | 1      | 198,10  | 198,10 | 5,23*   |
| F × H                                                                | 6      | 18,38   | 3,06   | 5,37**  |
| F × R                                                                | 6      | 64,61   | 10,77  | 18,90** |
| F × Tus                                                              | 3      | 13,85   | 4,62   | 8,11**  |
| H × R                                                                | 4      | 4,64    | 1,16   | 2,04    |
| H × Tus                                                              | 2      | 63,60   | 31,80  | 55,89** |
| R × Tus                                                              | 2      | 5,24    | 2,62   | 4,60*   |
| F × H                                                                | 12     | 21,61   | 1,80   | 3,16*   |
| F × H × Tus                                                          | 6      | 2,94    | 0,49   | < 1     |
| F × R × Tus                                                          | 6      | 13,66   | 2,28   | 4,00*   |
| H × R × Tus                                                          | 4      | 3,82    | 0,96   | 1,68    |
| F × H × R × Tus                                                      | 12     | 4,87    | 0,57   |         |

1) D.F. = Degré de liberté; S.S. = somme des carrés; M.S. = moyenne des carrés; F = variance; \* = significatif; \*\* = très significatif.

TABLEAU D'ANALYSE DE VARIANCE COMPLEXE DES MESURES MICROCALORIMETRIQUES, DES TRAITEMENTS AUX ULTRA-SONS

| Integra-<br>tion des<br>courbes<br>(Heures) | Traitement<br><br>R        | Traitement aux Ultra-Sons-Tus-   |                                 |                                 |                                  |                                   |                               |                               |                               |                                |                                  | Totaux                            |
|---------------------------------------------|----------------------------|----------------------------------|---------------------------------|---------------------------------|----------------------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|----------------------------------|-----------------------------------|
|                                             |                            | H <sub>2</sub> O + U.S.          |                                 |                                 |                                  |                                   | H <sub>2</sub> O              |                               |                               |                                |                                  |                                   |
|                                             |                            | Fréquences F                     |                                 |                                 |                                  | Som.                              | Fréquences F                  |                               |                               |                                | Som.                             |                                   |
|                                             |                            | 30                               | 80                              | 720                             | 960                              |                                   | 30                            | 80                            | 720                           | 960                            |                                  |                                   |
| 10                                          | 10<br>15<br>20<br><br>Som. | 2,80<br>3,75<br>3,50<br>10,05    | 2,34<br>2,50<br>2,42<br>7,26    | 2,50<br>2,19<br>2,80<br>7,49    | 1,39<br>4,30<br>3,02<br>8,71     | 9,03<br>12,74<br>11,74<br>33,51   | 1,78<br>2,25<br>0,71<br>4,74  | 1,70<br>2,40<br>1,12<br>5,22  | 0,55<br>1,53<br>2,40<br>4,48  | 0,66<br>2,42<br>2,39<br>5,47   | 4,69<br>8,60<br>6,62<br>19,91    | 13,72<br>21,34<br>18,36<br>53,42  |
| 20                                          | 10<br>15<br>20<br><br>Som. | 6,74<br>7,75<br>8,10<br>22,59    | 5,40<br>5,63<br>3,02<br>14,05   | 5,05<br>3,55<br>5,75<br>14,35   | 6,08<br>8,80<br>5,86<br>20,74    | 23,27<br>25,73<br>22,73<br>71,73  | 3,18<br>3,72<br>1,35<br>8,25  | 3,04<br>4,26<br>1,82<br>9,12  | 0,85<br>2,36<br>3,38<br>6,59  | 1,68<br>5,90<br>3,06<br>10,64  | 8,75<br>16,24<br>9,61<br>34,60   | 32,02<br>41,97<br>32,34<br>106,33 |
| 30                                          | 10<br>15<br>20<br><br>Som. | 13,80<br>15,60<br>14,90<br>44,30 | 13,05<br>12,15<br>7,50<br>32,70 | 12,20<br>8,02<br>12,50<br>32,72 | 14,00<br>16,70<br>12,40<br>43,10 | 53,05<br>52,47<br>47,30<br>152,82 | 8,35<br>8,30<br>6,65<br>23,30 | 8,10<br>8,10<br>4,80<br>21,00 | 2,82<br>5,00<br>7,60<br>15,42 | 5,10<br>13,00<br>6,30<br>24,40 | 24,37<br>34,40<br>25,35<br>84,12 | 77,42<br>86,87<br>72,65<br>236,94 |
| Totaux                                      |                            | 76,94                            | 54,01                           | 54,56                           | 72,55                            | 258,06                            | 36,29                         | 35,34                         | 26,49                         | 40,51                          | 138,63                           | 396,69                            |

On comprendra que, sous l'action des ultra-sons, tout ce système entre en vibration, la vitesse relative acquise par une particule dépendant de sa masse, de la fréquence et d'un coefficient de résistance dans le liquide (PRAT 1951).

Le fait que le phénomène de l'accélération de la germination continue à se manifester longtemps (plusieurs semaines ou plusieurs mois) après le traitement pourrait s'expliquer par des modifications structurales permanentes provoquées par le traitement ultra-sonore, ces effets étant liés à la durée du traitement et aux fréquences employées.

L'ensemble de ces résultats nous incite à penser qu'il n'y a pas eu qu'une modification des membranes, mais probablement des effets mécaniques et biochimiques plus profonds et dont l'influence se manifeste (au cours de la croissance) par une accélération générale du métabolisme (JOHNSON, OBOLENSKY 1954).

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L'adresse de la famille de l'auteur: †Dr. G. Obolensky, 5, rue St. Louis en l'Île, Paris 4ème.

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## Микрокалориметрическое изучение ускорения прорастания семян озвученных ультразвуком

Жорж Оболонски

Факультет естествознания, Париж, Факультет естествознания, Марсель

### Резюме

Семена ячменя (сорта «Фрея» и «Рита») озвучивались в течение 5—25 мин. разными частотами (30, 80, 570, 720 и 960 kHz) ультразвука интенсивности 2—5 W/cm<sup>2</sup>. При помощи микрокалориметрических измерений изучалась кинетика термогенеза и сравнивались кривые, полученные для опытных и контрольных семян. Установлено, что время физико-химического термогенеза (прием воды, набухание коллоидов итд.) в случае опытных семян значительно понижается. В дальнейшем наступает быстрый переход из физико-химической фазы в фазу биологического термогенеза, который даже может превышать фазу физико-химическую. Следовательно, действие ультразвука в зависимости от времени действия и частоты вызывает общее ускорение метаболических процессов, проявляющихся в ускорении прорастания. Данное явление необратимо и имеет место в течение нескольких недель и месяцев после озвучивания.

## BRIEF COMMUNICATION

## The Effect of Some Pyrimidine Derivatives on the Multiplication of Tobacco Mosaic Virus

MARIE ULRYCHOVÁ-ZELINKOVÁ

Department of Plant Pathology, Institute of Biology, Czechoslovak Academy of Sciences, Praha

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**Souhrn.** V rámci studia antimetabolitů virových nukleoproteinů u rostlin byl testován účinek 17 derivátů pyrimidinu proti viru tabákové mosaiky. Účinnost látek byla sledována v diskových kulturách. Preparáty byly zkoušeny v koncentraci 500  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$  a případně 5  $\mu\text{g/ml}$ . Výsledky byly statisticky zhodnoceny. Nejvyšší antivirový účinek vykázal 2-amino-4-methyl-6-chlorpyrimidin. V koncentraci 500  $\mu\text{g/ml}$  inhiboval reprodukci VTM z 98 %. Tento preparát měl inhibiční účinek ještě v koncentraci 100krát nižší. Na druhém místě stál strukturně blízký 2-amino-4-methyl-6-hydroxypyrimidin. Tento preparát inhiboval reprodukci VTM ze 77 %. Uracil v koncentraci 500  $\mu\text{g/ml}$  stimuloval reprodukci VTM o 36 %. Stimulační účinek 30 % měl také ethylester kyseliny S-(4-methyl-6-hydroxypyrimidyl-2)-thioglykolové. Toxický účinek proti listovým diskům tabáku v koncentraci 500  $\mu\text{g/ml}$  měly 2,6-dichlorpyrimidin, 2,6-dichlor-4-methylpyrimidin, 2,4,6-trihydroxypyrimidin, 2-thio-4,6-dihydroxypyrimidin, 2,4-dichlorpyrimidin a 2,6-dichlor-4-trichlormethylpyrimidin. V desetkrát nižší koncentraci působil na listové disky toxicky 2,4,6-trichlorpyrimidin.

**Summary.** The effect of 17 pyrimidine derivatives on tobacco mosaic virus was tested in leaf disk cultures. The greatest antiviral effect was found with 2-amino-4-methyl-6-chloropyrimidine, which in a concentration of 500  $\mu\text{g/ml}$  caused a 98% inhibition of TMV multiplication. The structurally closely related 2-amino-4-methyl-6-hydroxypyrimidine gave a 77% inhibition. An equal concentration of uracil caused a 36% stimulation of TMV multiplication. Also the ethyl ester of S-(4-methyl-6-hydroxypyrimidyl-2)-thioglycolic acid had a 30% stimulatory effect. The following preparations were toxic in the concentration mentioned above: 2,6-dichloropyrimidine, 2,6-dichloro-4-methylpyrimidine, 2,4,6-trihydroxypyrimidine, 2-thio-4,6-dihydroxypyrimidine, 2,4-dichloropyrimidine, 2,4,6-trichloropyrimidine, and 2,6-dichloro-4-trichloromethylpyrimidine.

**Introduction.** The effect of some pyrimidine derivatives was tested in connection with the examination of the anti-metabolites of virus nucleoproteins in plants. In spite of the fact that the mechanism of the effect of these analogues, which retard the synthesis of nucleic acids by being incorporated in place of the naturally occurring pyrimidine bases (MATHEWS and SMITH 1955), is today well known, it is not possible to foretell which of the derivatives will be effective and against which virus disease. A better understanding of the structure and type of new effective anti-metabolites can contribute to a more thorough conception of the biosynthesis of nucleic acids both in hosts and viruses.

It was from this point of view that the effect of 17 pyrimidine derivatives on tobacco mosaic virus multiplication was tested. The preparations were synthesised by Dr. Z. Müller at the Research Institute for the Technology of Agricultural Chemistry in Bratislava.

**Material and Method.** The efficacy of the substances was followed in leaf disk cultures (COMMONER and MERCER 1951, SCHLEGEL and RAWLINS 1954). Leaves of *Nicotiana tabacum*

Table 1

| Preparation                                                                                                  | Conc.<br>$\mu\text{g./ml.}$ | Inhibition<br>%     | Number of lesions on half leaf of<br><i>N. glutinosa</i><br>Average and mean error |                     | P                    | n  |
|--------------------------------------------------------------------------------------------------------------|-----------------------------|---------------------|------------------------------------------------------------------------------------|---------------------|----------------------|----|
|                                                                                                              |                             |                     | Control                                                                            | Treated             |                      |    |
| 2-amino-4-hydroxypyrimidine                                                                                  | 500                         | 10.4                | 29.2 $\pm$ 3. 6.2                                                                  | 26.4 $\pm$ 3. 5.3   | < 0.7                | 15 |
| 2-amino-4-methyl-6-hydroxypyrimidine                                                                         | 500                         | 77.1                | 19.5 $\pm$ 3. 2.3                                                                  | 4.4 $\pm$ 3. 0.5    | < 10 <sup>-10</sup>  | 15 |
| 2-amino-4-methyl-6-chloropyrimidine                                                                          | 500                         | 98.2                | 19.8 $\pm$ 3. 2.5                                                                  | 0.35 $\pm$ 3. 0.13  | < 10 <sup>-10</sup>  | 20 |
| 2-amino-4-methyl-6-chloropyrimidine                                                                          | 50                          | 50.7                | 363.0 $\pm$ 3. 39.9                                                                | 179.0 $\pm$ 3. 26.4 | < 10 <sup>-3</sup>   | 10 |
| 2-amino-4-methyl-6-chloropyrimidine                                                                          | 5                           | 36.4                | 184.5 $\pm$ 3. 26.5                                                                | 117.3 $\pm$ 3. 11.0 | < 2.10 <sup>-2</sup> | 10 |
| 2-amino-4,6-dihydroxypyrimidine                                                                              | 500                         | 20.1                | 203.7 $\pm$ 3. 25.2                                                                | 162.7 $\pm$ 3. 23.5 | < 0.2                | 15 |
| 2-amino-2,6-dihydroxypyrimidine                                                                              | 500                         | 6.0                 | 214.3 $\pm$ 3. 18.3                                                                | 201.4 $\pm$ 3. 18.9 | < 0.5                | 10 |
| 2,6-dihydroxypyrimidine = uracil                                                                             | 500                         | 36.6 st,<br>lethal* | 267.7 $\pm$ 3. 25.8                                                                | 366.4 $\pm$ 3. 37.6 | < 2.10 <sup>-2</sup> | 15 |
| 2,6-dichloropyrimidine                                                                                       | 500                         | 15.9                | 189.7 $\pm$ 3. 23.6                                                                | 159.5 $\pm$ 3. 21.9 | < 0.3                | 10 |
| 2,6-dichloro-4-methylpyrimidine                                                                              | 50                          | 23.3                | 12.3 $\pm$ 3. 2.6                                                                  | 9.4 $\pm$ 3. 1.5    | < 0.3                | 15 |
| 2,6-dichloro-4-methylpyrimidine                                                                              | 500                         | lethal              | 10.3 $\pm$ 3. 1.9                                                                  | 4.9 $\pm$ 3. 1.1    | < 2.10 <sup>-2</sup> | 10 |
| 2,6-dichloro-4-methylpyrimidine                                                                              | 50                          | lethal              |                                                                                    |                     |                      |    |
| 2,4,6-trihydroxypyrimidine                                                                                   | 50                          | 0                   |                                                                                    |                     |                      |    |
| 2,4,6-trihydroxypyrimidine                                                                                   | 50                          | lethal              |                                                                                    |                     |                      |    |
| 2-thio-4,6-dihydroxypyrimidine                                                                               | 500                         | 38.7                | 8.0 $\pm$ 3. 2.8                                                                   | 4.9 $\pm$ 3. 1.0    | < 0.3                | 10 |
| 2-thio-4,6-dihydroxypyrimidine                                                                               | 50                          | 6.3                 | 248.6 $\pm$ 3. 27.3                                                                | 232.8 $\pm$ 3. 23.8 | < 0.5                | 10 |
| 2-thio-4-methyl-6-hydroxypyrimidine<br>ethyl ester of S-(4-methyl-6-hydroxy-<br>pyridyl-2)-thioglycolic acid | 500                         | 30.2                | 127.5 $\pm$ 3. 14.1                                                                | 166.1 $\pm$ 3. 16.6 | = 5.10 <sup>-2</sup> | 10 |
| 2,4-dichloropyrimidine                                                                                       | 500                         | lethal              |                                                                                    |                     |                      |    |
| 2,4-dichloropyrimidine                                                                                       | 50                          | 3.5                 | 50.3 $\pm$ 3. 7.6                                                                  | 48.5 $\pm$ 3. 4.5   | < 0.8                | 10 |
| 2,4,6-trichloropyrimidine                                                                                    | 500                         | lethal              |                                                                                    |                     |                      |    |
| 2,4,6-trichloropyrimidine                                                                                    | 50                          | lethal              |                                                                                    |                     |                      |    |
| 2,6-dichloro-4-trichloromethylpyrimidine                                                                     | 500                         | 10.0                | 2.5 $\pm$ 3. 1.2                                                                   | 2.2 $\pm$ 3. 0.4    | < 0.8                | 10 |
| 2,6-dichloro-4-trichloromethylpyrimidine                                                                     | 50                          |                     | 52.2 $\pm$ 3. 5.6                                                                  | 42.0 $\pm$ 3. 6.0   | < 0.2                | 15 |
| 2,6-dihydroxy-4-carboxy-5-aminopyri-<br>midine                                                               | 500                         | 19.5                |                                                                                    |                     |                      |    |

\* The leaves lose turgor, become glassy and brown.

Samsun were removed from the plants, dusted with carborundum powder and rubbed with non-diluted crude infective sap. The leaves were then washed with running tap water and disks were punched out with a cork-borer, those from one half-leaf being used for the control, from the other for the experiment. The control disks were laid with the upper, i.e. inoculated sides on the surface of pure water in a Petri dish, while the treated disks floated on a solution of the substance being tested. The area of one disk was 1 cm<sup>2</sup>. There were 70 disks in each dish. The Petri dishes were put into a thermostat at 30° C with continuous illumination by two fluorescent tubes at a distance of 20 cm. and with light flow of 1500 Lm (approx. 600 Lux). On the fourth day the disks were thoroughly rinsed, water was removed with cotton wool and the disks were homogenized in a mortar with 2 ml. of water. The homogenate was then heated for 12 min. in a water bath at 67° C and following centrifugation the clear supernatant was used for a quantitative test on *Nicotiana glutinosa*. Pyrimidine derivatives were tested in concentrations of 500 µg./ml., 50 µg./ml. and in some cases 5 µg./ml. The experiments were started in May and continued to September 1959.

**Results and Discussion.** The results are summarised in the table. The greatest antiviral effect was exerted by 2-amino-4-methyl-6-chloropyrimidine. In a concentration of 500 µg./ml. it inhibited TMV multiplication by 98%. This preparation still had an inhibitory effect in a concentration of 5 µg./ml. The structurally closely related 2-amino-4-methyl-6-hydroxypyrimidine showed a 77% inhibitory effect on TMV multiplication. The remaining preparations failed to show any significant antiviral effect. Uracil at a concentration of 500 µg./ml. stimulated TMV multiplication by 36.6%. The ethyl ester of S-(4-methyl-6-hydroxypyrimidyl)-2-thioglycolic acid caused 30.2% stimulation. The following substances in concentration 500 µg./ml. had a toxic effect on tobacco leaf disks: 2,4-dichloropyrimidine, 2,6-dichloro-4-methylpyrimidine, 2,4,6-trihydroxypyrimidine, 2-thio-4,6-dihydroxypyrimidine, 2,4-dichloropyrimidine, 2,4,6-trichloropyrimidine and 2,6-dichloro-4-trichloromethylpyrimidine. At concentrations ten times lower, 2,4,6-trichloropyrimidine had a toxic effect on the leaf disks and 2,6-dichloro-4-methylpyrimidine acted as an antiviral agent, retarding TMV multiplication by 52%.

The results show that derivatives with two or three halogens had toxic effect on the leaf disks. However, LINDNER et al. (1959) in their most recent work, which appeared after the completion of the present investigation, also tested 2,4,6-trichloropyrimidine applying it in the form of a spray and did not observe any harmful effects. On the other hand, it seems that the presence of chlorine is to a great degree responsible for the strong effect of the preparation. For example, SCHLEGEL and RAWLINS (1954), using the same leaf disk culture method, found that 2-amino-4-methylpyrimidine inhibits TMV multiplication in concentration of 100 µg./ml. by 32%. The 2-amino-4-methyl-6-chloropyrimidine used in the present experiments caused 98% inhibition when used in 500 µg./ml. concentration and in a concentration of 5 µg./ml. the inhibitory effect was still 36%.

**Acknowledgment:** The author wishes to express her sincere thanks to the Research Institute for Technology of Agricultural Chemistry in Bratislava and to Dr. Z. Müller for providing material for the experiments.

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*Address:* Dr. Marie Ulrychová-Zelinková, Fytopatologické odd. Biologického ústavu ČSAV Na Karlovce 1, Praha 6 - Dejvice.

BIOL. PLANT. 2 (3) : 240—243, 1960

## Влияние некоторых производных пириимидина на репродукцию вируса табачной мозаики

Марие Улрыхова-Зелинкова

Отделение фитопатологии Биологического института Чехословацкой Академии Наук,  
Прага

### Резюме

В связи с изучением антиметаболитов вирусных нуклеопротеидов у растений изучалось противовирусное действие 17 производных пириимидина на вирус табачной мозаики (ВТМ). Действие препаратов исследовалось методом дисковых культур. Препараты испытывались в концентрациях 500  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$  и 5  $\mu\text{g/ml}$ . Результаты опытов обрабатывались статистически. Наивысшее антивирусное действие установлено в случае 2-амино-4-метил-6-хлорпириимидина. В концентрации 500  $\mu\text{g/ml}$  тормозил репродукцию ВТМ на 98%. Препарат обнаружил тормозящее действие еще в концентрации в 100 раз пониженной. На втором месте по своему противовирусному действию стоял структурно близкий 2-амино-4-метил-6-гидроксипириимидин. Этот препарат тормозил репродукцию ВТМ на 77%. Урацил в концентрации 500  $\mu\text{g/ml}$  стимулировал репродукцию ВТМ на 36%. Стимулирующее действие в 36% выявлено также в случае этилэфира S-4-метил-6-гидроксипириимидин-2/-тиогликолевой кислоты. Токсическое действие на диски листьев табака в концентрации 500  $\mu\text{g/ml}$  установлено в случае 2-6-дихлорпириимидина, 2,4,6-тригидроксипириимидина, 2,6-дихлор-4-метилпириимидина, 2-тио-4,6-дихлорпириимидина, 2,4,6-трихлорпириимидина и 2,6-дихлор-4-трихлорметилпириимидина. В концентрации в 10 раз пониженной действовал токсически на диски листьев 2,4,6-трихлорпириимидин.

Выражаем благодарность д-ру инж-у З. Муллеру из Научно-исследовательского ин-та агрономической техники в Братиславе за любезное предоставление нам препаратов.

## BRIEF COMMUNICATION

***Chenopodium Quinoa* Willd. as a Local Lesion Host of  
*Marmor cichorii* Kvíčala (*Cichorium virus* 1 Kvč.)**

JAROSLAV BRČÁK

Department of Plant Pathology, Biological Institute, Czechoslovak Academy of Sciences, Praha

Received May 20, 1960

**Souhrn:** Kvíčalou (1956) popsaný virus skvrnitosti a žloutenky čekanky, známý pouze z *Cichorium intybus* L. var. *sativum* Bl. byl mechanicky přenesen na *Chenopodium Quinoa* Willd., na jehož listech vyvolává žluté lokální nekrotické lese. Lese jsou podobné jako u 6 srovnávaných kmenů viru obyčejné okurkové mosaiky [*Cucumis virus* 1 (Doolittle) Smith, *Marmor cucumeris* var. *vulgare* Holmes], avšak projeví se za delší dobu po očkování (9 dní).

**Summary:** Chicory blotch and yellows virus caused yellow local necrotic lesions on the leaves of *Chenopodium Quinoa* within nine days. Six strains of the common cucumber mosaic virus gave similar lesions in all cases, but in a shorter incubation period.

**Introduction:** Kvíčala (1956) described a new virus disease of chicory (*Cichorium intybus* L. var. *sativum* Bl.) as chicory blotch and yellows. This virus has hitherto been diagnosed only by transmission to chicory. The virus causes vein clearing followed by mottling, yellow blotch, necrosis and leaf deformation in chicory and is sap transmissible. Kvíčala's attempts to transmit the virus by *Myzus persicae* Sulz. and *Aphis fabae* Scop. failed. Negative results were obtained in experiments made to transmit the virus by mechanical inoculation to five solanaceous plants as well as to lettuce. The incubation period of the virus was 14—54 days, the dilution end point 1 : 50, the 10 min. inactivation temperature between 65 and 70° C, and the longevity *in vitro* did not last more than 24 hours. The first local lesion host of this virus is described in the present paper.

**Material and Methods:** One infected plant of chicory (from dr. Kvíčala's experiments) was used as the source of infection. Healthy *Ch. Quinoa* seedlings were used as the test plants. Half-leaves powdered with Carborundum (600 mesh) were inoculated with crude undiluted sap by a glass spatula. Sap taken from healthy chicory seedlings was used for the control inoculations of the opposite half-leaves. All experiments were carried out in glasshouse conditions in April and May 1960. The test plants were kept during the incubation period at an average temperature of 24° C (av. min. 7° C, av. max. 36° C) and at an average relative humidity of 64% (av. max. 96%, av. min. 38%).

**Results:** The shortest incubation period obtained in chicory seedlings was 33 days. In *Ch. Quinoa* the infection resulted in the production of faint yellowish local lesions nine days after inoculation. Lesions enlarged to 1.5—2.5 mm. in diameter in the next five days and became necrotic, especially on the lower leaves. They were well distinct on both sides of the leaf edge. At the same time six different strains of the common cucumber mosaic virus were compared with the virus mentioned above. All of them caused lesions on *Ch. Quinoa* like those of the chicory virus, but the incubation period of the latter was nearly twice as long as with all strains of the common cucumber mosaic virus. In spite of these results *Ch. Quinoa* is likely not to differentiate between the two viruses.



Fig. 1. Local lesions on the leaves of *Chenopodium Quinoa* caused by the chicory blotch and yellows virus—14 days after inoculation (Orig. J. Brčák).

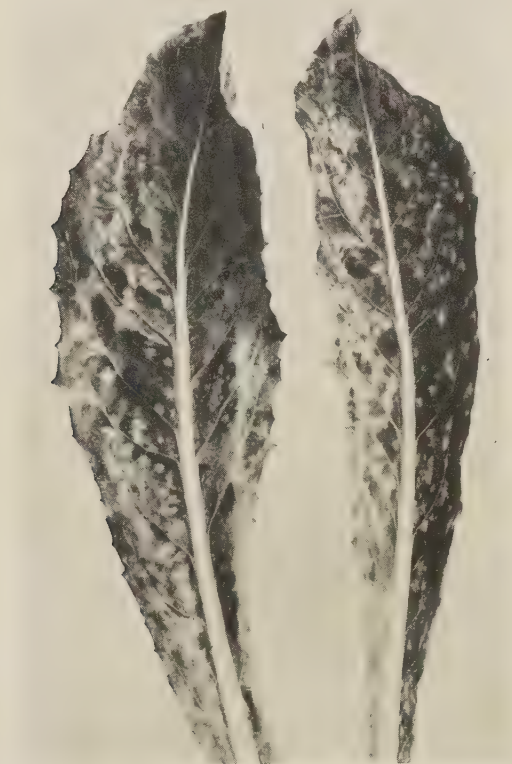


Fig. 2. Symptoms of chicory blotch and yellows virus in *Cychorium intybus* var. *sativum*. (Orig. J. Brčák)



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Address: C. Sc. RNDr. Jaroslav Brčák, Fytopatologické odd. Biologického ústavu ČSAV, Praha 6-Dejvice, Na Karlovce 1.

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BIOL. PLANT. 2 (3) : 244—245, 1960

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***Chenopodium Quinoa* Willd. как растение - индикатор вируса цикории  
(*Marmor cichorii* Kvíčala, *Cichorium virus* 1 Kvč.)**

Ярослав Брчак

Отделение фитопатологии Биологического института Чехословацкой Академии Наук,  
Прага

## Резюме

Квичалой (1956) описанный вирус мозаичного заболевания цикории, известный только у *Cichorium intybus* L. var. *sativum* Bl., был механически перенесен на *Chenopodium Quinoa* Willd., на листьях которого он вызывает желтые местные некрозы. Местные некрозы похожи, как у 6-и сравниваемых штаммов вируса обыкновенной огуречной мозаики (*Cucumis virus* 1 (Doolittle) Smith, *Marmor cucumeris* var. *vulgare* Holmes), но они проявятся через более длительный период после инокуляции (9 дней).

## METHODICA

## Staining of Chromosomes in Root Tips of Clover *in toto* with Aceto-galloyanine

Jiří BARTOŠ and NADĚŽDA AVRATOVŠČUKOVÁ

Botanical garden of the Slovak Academy of Sciences, Košice

Received February 16, 1960

**Souhrn.** Autoři popisují rychlou cytologickou metodu barvení chromosomů v kořenových špičkách jetele, založenou na použití roztoku galloyaninového chromového laku ve zředěné kyselině octové.

Standard methods of obtaining rapid preparations of root tips, using acetocarmine, acetoorcein, acetolacmoid, acetonigrosine, do not produce sufficiently intense and clear pictures of chromosomes in the root tips of clover. For chromosome counting and for orientation studies of chromatin structures in clover root tips a method can be recommended which employs a solution of galloyanine in chromium mordant in dilute acetic acid for staining.

|                    |                                           |         |
|--------------------|-------------------------------------------|---------|
| Procedure:         | Fixative: 1% aqueous trichloroacetic acid | 100 ml. |
|                    | 5% aqueous chromium-potassium sulphate    | 20 ml.  |
|                    | concentrated sulphuric acid               | 2 ml.   |
| Aceto-Galloyanine: | glacial acetic acid                       | 45 ml.  |
|                    | 5% aqueous chromium-potassium sulphate    | 55 ml.  |
|                    | galloyanine                               | 2 g.    |

The mixture is boiled under a reflux condenser and filtered after cooling. After a certain time the dye solution is found to contain a precipitate which must be removed by filtration. The solution can be used for several months.

### Preparation technique:

Root tips of clover are fixed in the above fixative for 12 to 24 hours, when chromatin structures are simultaneously fixed and mordanted and the tissue becomes partly macerated. Then the fixed objects are transferred directly to the acetogalloyanine. Within 12 to 24 hours the objects are sufficiently stained; no overstaining occurs on prolonged keeping in the mordant. The blue-black stained root tips are transferred to a drop of the aceto-galloyanine on a microscope slide, covered with a cover-glass and crushed by gentle pressure. The preparation is now turned over and placed on two glass rods in a Petri dish containing 100% tertiary butanol; the cover-glass is then left to drop off the slide. Both the slide and the cover-glass with adhering cells are passed through pure benzene and the preparation is mounted in dammar resin.

Result of staining: Chromatin structures are stained blue to blue-black, other structures are either colourless or have a light grey tinge.

Иржи Бартош, Надежда Авратовщуклова

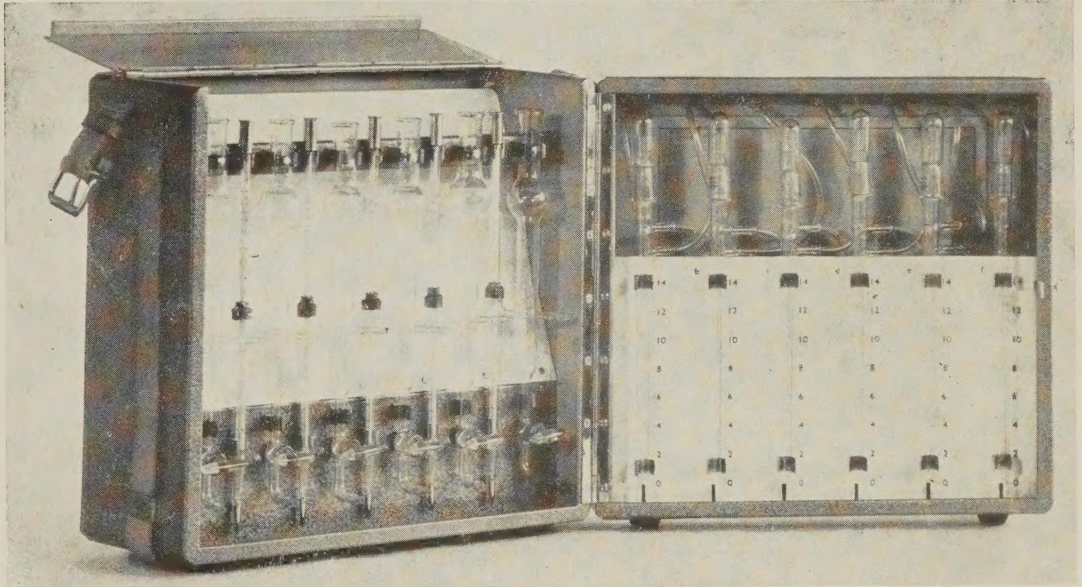
### Окраска хромосом в кончиках корня клевера *in toto* ацетогаллоцианином

Приводится описание по личному опыту авторов вполне для краски хромосом кончиков корней клевера подходящего сквеч метода, основанного на применении раствора галлоцианинового хромового лака в разбавленной уксусной кислоте.

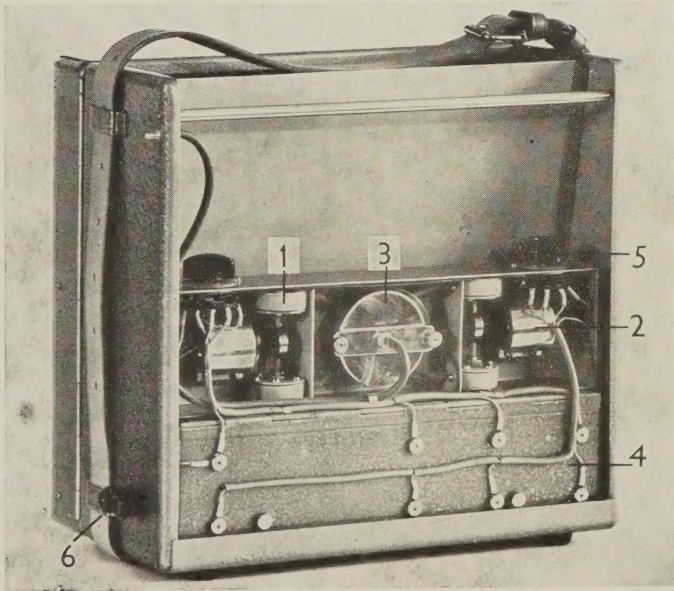
## Полевой прибор для определения интенсивности фотосинтеза

BIOLOGIA PLANTARUM 2(2) : 107—112, 1960

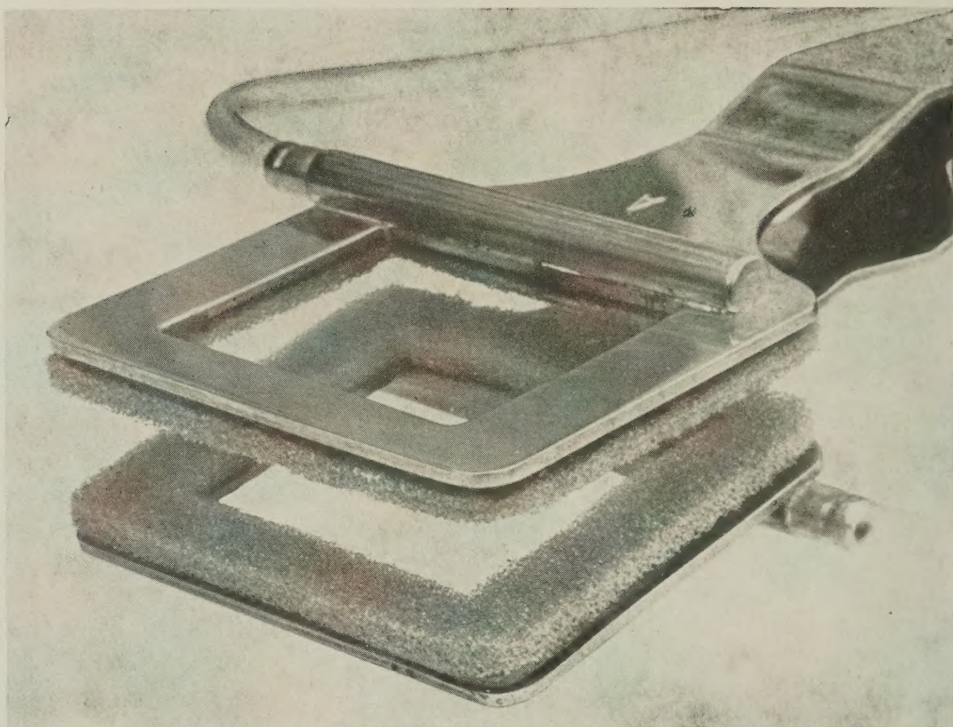
Следующие фотографии являются дополнением к указанной работе.



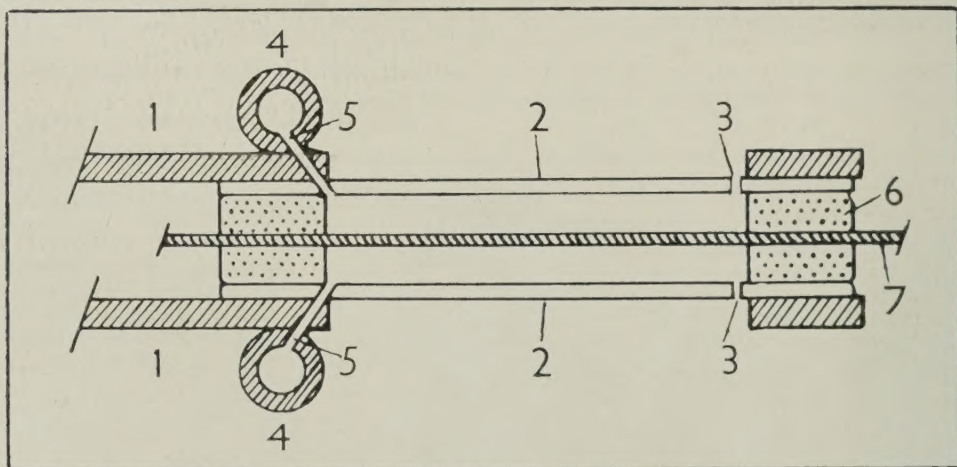
1) Новый шестиместный тип прибора после открытия передней крышки с кассетными реометрами (вправо).



2) Задняя сторона прибора после снятия крышки: легко вынимаемый блок состоящий из четырех мембранных насосиков (1)двигаемых с помощью двух электромоторов в 12 вольт постоянного тока (2). Среди насосиков находится сосуд со силикагелем для сушки воздуха (3). Внизу находится футляр для 20 карманных батареек по 4,5 вольт (4),включаемых в зависимости от требуемой мощности последовательно переключателями (5). Блок насосиков с источниками питания можно, конечно, использовать самостоятельно и для других полевых работ. По стороне прибора находятся контактные гнезда (6) для включения добавочного трансформатора и для выпрямителя в случае питания прибора от сети. Добавочный трансформатор и выпрямитель находятся в коробке из искусственного материала размерами в 130 × 90 × 60 мм.



3) Модификация камеры-щипцов по Ничипоровичу (1955).



- 4) Поперечное сечение камерой-щипцами, изготовленной из хромированной латуни (1) с окошками из плексигласа (2). Воздух входит в камеру отверстиями 3 и отсасывается трубками 4 с отверстиями 5. Эти отверстия от начала трубки постепенно увеличиваются и тем обеспечивают равномерный ток воздуха через камеру. Контрольный воздух отсасывается отдельно. Для теснения листа используется полиуретановая пена (6), которая надежно обеспечивает теснение и самых сильных нервов у листьев (7). Проницаемость пористой полиуретановой пены является по сравнению с проницаемостью вступительных отверстий столь ничтожной, что практически весь отсасываемый воздух проходит этими отверстиями. Однако исходя из результатов КОСН-а (1956) полное теснение не является необходимым, если обеспечено отсасывание всего воздуха обедненного  $\text{CO}_2$  листьем.

Časopis pro experimentální botaniku  
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- I. HRŠEL, J. JURÁKOVÁ, K. BENEŠ: Cytochemical and Electron-Microscopic Investigation of the Golgi-Apparatus and Golgi-Resembling Inclusions in the Root Meristem of *Fagopyrum esculentum* Moench
- F. PLHÁK: Veränderungen des Wachstums und der Atmungsvorgänge von Maispflanzen, hervorgerufen durch erhöhte Dichte der Bestände
- V. PRŮŠA, I. VACEK: Wheat Striate Virus
- I. CETL: The Size of Pollen Grain of Genus *Triticum* L.
- I. ŠETLÍK, J. BARTOŠ, Š. KUBÍN: Photosynthesis in Leaf Disks as a Measure of Photosynthetic Capacity in crop Plants
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- B. SLAVÍK: Ecophysiological Significance of the Height Gradient of the Equivalent Evaporation Power of the Atmosphere
- V. PRŮŠA, J. VACEK: Transmission of Wheat Striate Virus by the Eggs of the Vector *Calligypona pellucida* Fabr.
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- Л. Хвойка: Влияние плодов на осмотическое давление клеточного сока листьев и закладку цветочных почек у яблони

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